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INTRAVENOUS AUTOHEMOTHERAPY WITH HEMOLYZED BLOOD: TECHNIQUE AND LEUCOPOIETIC RESPONSE

PRELIMINARY REPORT*

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INTRODUCTION

THE present report, while complete in itself, is intended to serve as an introduction to a more intensive study concerning the mechanism of malaria inoculata in neurosyphilis, the principal text of which will be reported separately.

The modern vogue of treating neurosyphilis with mechanical hyperpyrexia has focused the attention of investigators, with renewed emphasis, upon the question of whether the beneficial results in these cases are dependent upon fever per se or upon fever plus a biologic factor such as inheres in malaria. Any study of the mechanism of malaria must take into account the fundamental constituents which go to make it up. Of these there are at least three, each of which is apparently capable of provoking a defensive reaction in the organism. These are (1) the malarial parasite, (2) fever, and (3) blood destruction.

One of these—fever—we can already duplicate at will. We reasoned that if we could introduce another constituent, such as the factor of blood destruction, we might approach a step nearer to the artificial duplication of malaria. It seemed logical to attempt this by giving a group of patients intravenous injections of hemolyzed autogenous blood just prior to the induction of fever; al-

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though, as far as we know, no one before has attempted to break down the problem in just this manner. Various pertinent observations were then planned which would lend themselves to comparison with certain results known to be typical of malaria. Our first investigation was directed toward the alterations in the cellular equilibria of the organism. For this purpose each patient was observed during four separate periods, of which this report concerns itself only with the first two: (1) A control or basal period, (2) a period of autohemotherapy alone, (3) a period of pyretotherapy alone, and (4) a period of autohemopyretotherapy; this is a term coined by us to conveniently designate the combination of autohemotherapy and induced fever. We hope, incidentally, that those investigators who are familiar with the excellent results sometimes obtained with autohemotherapy in various morbid processes, will recognize in combined autohemopyretotherapy a new therapeutic and experimental approach to the problems of enhancing the defensive mechanism of the organism. Although a considerable literature has accumulated about autohemotherapy, surprisingly little has been written about its intravenous use, especially with hemolyzed blood.

Descarpentries[1, 2] employs distilled water and blood in a proportion of 2:1, lysing 10 c.c. uncitrated blood in 20 c.c. distilled water; he then agitates the mixture in a flask containing several marbles, about which the clumps of fibrin adhere; finally, he injects the clear fluid subcutaneously in divided doses—a momentarily painful, but, according to him, a very beneficial procedure in various septic conditions. He believes that, among other effects, the hemopoietic system is stimulated, but offers no data in support of this view.

Zimmerman[3] cautiously adds 2 c.c. distilled water to 20 c.c. blood and immediately reinjects it either intravenously or intramuscularly. He states that there is *usually* a decrease in the total number of leucocytes, eosinophiles, and lymphocytes; but that the neutrophiles exhibit a relative increase. Frey[4] believes that lysis, following the intravenous injection of hemolyzed blood, is prolonged in the blood stream, but Zimmerman does not agree with that view.

Brunner and Breuer[5] place 14 c.c. distilled water into a 20 c.c. syringe, aspirate blood into the same syringe to its capacity, then, without drawing the needle out of the vein, reinject the mixture.

Thus it may be seen that there is considerable divergence of opinion regarding technique and physiologic results; one is led to believe that the choice of technique has been empirical rather than based upon an experimental foundation. In fact, we were able to obtain only very meager information in answer to the three fundamental questions that we had asked ourselves, viz.:

1. What is the average minimal hemolytic ratio of distilled water to blood?

2. How much hemolyzed autogenous blood can be injected intravenously, allowing a fair margin of safety?

3. What is the nature of the leucopoietic response, if any, to such injections?

We shall, therefore, endeavor to answer these and other concomitant questions as a result of our own investigations. In so doing, this report hopes to establish a rational basis for the technique of intravenous autohemotherapy, either experimental or therapeutic.

PROCEDURE

I. *Determination of Minimal Hemolytic Ratio.*—Graduated amounts of distilled water, ranging from 0.2 c.c. to 5 c.c., were placed in a series of test tubes. To each tube, 2 c.c. of venous blood were then added, using noncitrated blood in one series, and citrated blood in another series. The progress of hemolysis was then observed in each tube, both macroscopically and microscopically.

In the noncitrated series it was observed that hemolysis began when the proportion of water and blood was 1:1, but proceeded slowly and incompletely due to the fact that numbers of red blood cells became enmeshed in the fibrin clumps, where they were not so well exposed to the action of the distilled water.

In the citrated series a more favorable course of events took place. Here also the minimal hemolytic ratio was 1:1; but, there being no fibrin clumps, all the erythrocytes were exposed to the distilled water, with the result that hemolysis was much more rapid and complete.

Having determined that the minimal hemolytic ratio was approximately 1:1, we tried adding a little whole blood to a mixture of that proportion in order to see if any further hemolysis would occur; however, the added blood merely settled to the bottom of the tube without hemolyzing.

A practical point to keep in mind is this: When blood is mixed with the proper proportion of distilled water, hemolysis occurs very rapidly, and the mixture becomes fluid and homogeneous almost at once; but if allowed to stand for any length of time at room temperature, it begins to stiffen and jell slightly. Therefore, the optimum time to reinject the mixture is within ten minutes after mixing.

II. *Determination of Amount of Injection.*—We know from Ross's estimation of the number of parasites present during a malarial paroxysm, that a considerable amount of blood must be destroyed during each chill. It seemed to us therefore, that much larger amounts of lysed blood than indicated in the literature could be injected with impunity. Working first with rabbits, we found that they could easily tolerate an injection of as much as 52 c.c. of total solution, consisting of 20 c.c. blood, 2 c.c. citrate, and 30 c.c. distilled water.

Thus encouraged, we then proceeded with similar experiments in human beings. As stated above, the average minimal hemolytic ratio between blood and distilled water was found to be 1:1. In order to be perfectly accurate, one should, no doubt, determine the hemolytic ratio in each patient treated; however, in this type of experiment average values are adequate. We therefore decided to employ routinely a proportion of three parts distilled water to two parts blood, feeling that such a mixture would cause optimal hemolysis without too great an excess of distilled water in the majority of cases.

In all, seven patients were observed; their ages varied from 30 to 57 years. The diagnosis in each case was general paresis. Some had received fever therapy prior to the present investigation. These and other data are summarized in Table I since they may have a possible bearing upon the results obtained. Certain clinical data, such as temperature, pulse, blood pressure, and subjective sensations, were also recorded for several hours before and after the injections.

TABLE I

NAME AND GROUP	DIAGNOSIS	AGE	DURATION OF SYPHILIS	HOURS OF FEVER 103° TO 106°	AMOUNT OF INJECTION IN C.C.			
					BLOOD	CITRATE	DISTILLED WATER	TOTAL
H.F. (A)	General paresis	36	?	46	10	1	15	26
G.F. (A)	General paresis	32	12 yr.	Malaria	20	2	30	52
C.K. (B)	General paresis, optic atrophy, retinitis	57	(?) 18 yr.	46	30	3	45	78
C.C. (A)	General paresis	38	13 yr.	80	30	3	45	78
R.B. (A)	General paresis, cachexia	37	?	0	40	4	60	104
W.S. (B)	General paresis, 6th nerve palsy	50	(?) 18 yr.	28	50	5	75	130
S.E. (B)	General paresis, funnel chest, tachycardia	30	(?) 12 yr.	0	50	5	75	130
Average		40	(?) 15 yr.	35	33	3.3	50	86

The amounts of hemolyzed blood injected ranged from 10 c.c. to 50 c.c., the average being 33 c.c. The technique is quite simple. Into a 100 c.c. syringe is placed enough sodium citrate solution to make up 10 per cent of the amount of blood that is withdrawn from the vein. This citrated blood is left in the syringe, and, after sticking a sterile cork over the point of the needle to prevent leakage, is shaken vigorously for about five minutes. Then one and a half times as much distilled water is drawn into the same syringe to make a proportion of 3:2, and the mixture is again agitated for five minutes. Finally the entire solution is reinjected intravenously. If the total amount of the solution is more than the syringe can hold, the distilled water may be placed in a beaker and the blood mixed with it by swishing it back and forth through the syringe. Aside from warming the distilled water to body temperature and keeping the entire procedure aseptic, no other special precautions are necessary.

RESULTS

Not the slightest discomfort was experienced by any of the patients following the injection. In no case was there even the semblance of a chill or a sign of jaundice. The temperature and pulse rate were not affected. Blood pressures in a few cases showed transient changes, but these were so slight and inconstant that no significance has been attached to them.

III. The Leucopoietic Response. A. Method of Determination.—Before attempting to evaluate the leucocytic response to any pathologic or chemical disturbance, it is important first to determine an adequate *normal* base line derived from frequent successive readings, for it is now recognized that continual fluctuations occur in the white blood cells even under physiologic conditions. Accordingly the following method of comparison was adopted. Each patient was observed during an eight- or ten-hour period, usually between 8:00 A.M. and 3:00 to 5:00 P.M., on two successive days; the first day served as a control period, the second day a period of autohemotherapy. All other factors were kept as constant as possible. The patient was kept in bed and allowed a regular house

TABLE II

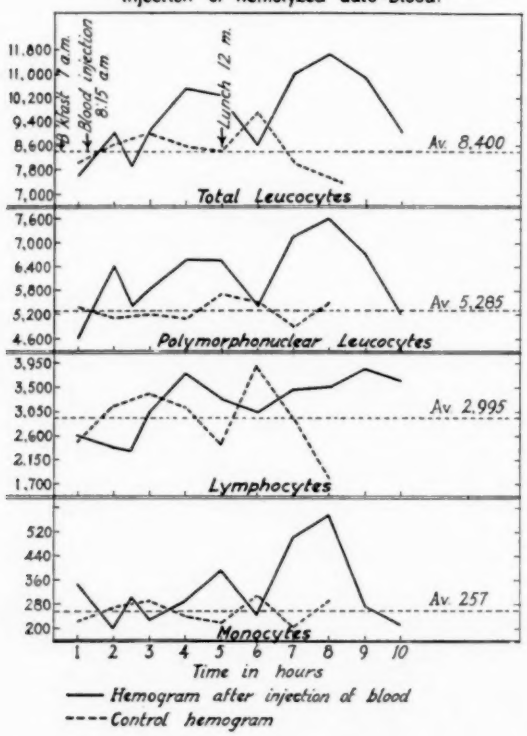
GROUP	PATIENT	TOTAL LEUCOCYTES			POLYMORPH. CELLS			LYMPHOCYTES			MONOCYTES		
		CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE	CONTROL	BLOOD INJECTION	PER CENT CHANGE
A (Leucocytosis)	R. B.	8,800	10,500	19	5,600	6,900	23	3,200	3,700	16	86	316	267
	G. F.	9,000	10,300	14	4,800	5,800	21	3,800	4,100	10	379	379	N.S.
	C. C.	8,400	9,500	13	5,700	6,500	14	2,500	2,600	N.S.	234	248	N.S.
	H. F.	7,900	8,700	10	4,900	6,000	23	2,600	2,600	N.S.	340	404	19
Average for Group A		8,500	9,800	14	5,200	6,300	21	3,000	3,200	N.S.	260	337	30
B (Leucopenia)	C. K.	8,800	7,100	19	5,400	3,900	28	3,100	2,900	N.S.	302	276	N.S.
	W. S.	8,400	5,200	38	5,500	3,100	42	2,800	2,100	25	95	35	63
	S. E.	9,300	7,800	17	5,400	4,300	20	3,700	3,500	N.S.	189	65	65
Average for Group B		8,800	6,700	25	5,400	3,800	30	3,200	2,860	13	195	125	40

diet. Blood counts and films were obtained at thirty- to sixty-minute intervals during each eight- to ten-hour period, using Bureau of Standards pipettes and counting chambers.

Altogether a total of 115 blood specimens were taken; 51 during the control periods and 64 during the injection periods. With the above facts in mind, we feel that the averages of our numerous readings afford a reasonably accurate criterion for estimating our results, and that any alteration of the basal average amounting to 10 per cent or more can be looked upon as the specific result of the autogenous blood injections.

CHART 1

GROUP A. Leucocytosis following intravenous injection of hemolyzed auto-blood.



B. Results.—We found that our patients fell into two groups: Group A (four patients) exhibited an *increase* in all the leucocytic elements following autohemotherapy. Group B (three patients) showed a *decrease*. The averages and percentage changes for each patient are summarized in Table II. Only changes of 10 per cent or more are recorded numerically, those below 10 per cent being marked N. S. meaning "not significant."

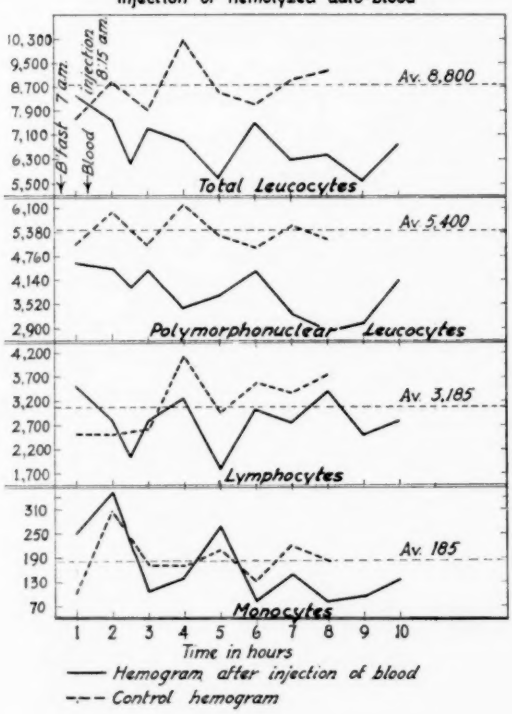
Taking up each element of the hemogram separately, first in Group A, it will be noticed that the total white cell count displays elevations, varying from 10 to 19 per cent; of the differential elements, the polymorphonuclear cells reveal the most consistent increases, varying from 14 to 23 per cent; the lymphocytes exhibit significant rises in two cases; while the monocytes develop the

greatest and most inconsistent fluctuations, varying from "not significant" in two cases to a tremendous increase of 267 per cent in one case. The *average* changes for the four patients are as follows: (1) total leucocytes 14 per cent increase, (2) polymorphonuclear cells 21 per cent increase, (3) lymphocytes "not significant," and (4) monocytes 30 per cent increase.

Considerably greater alterations occur in Group B. The reductions in the total number of white blood cells vary from 17 to 38 per cent; polymorphonuclears, from 20 to 42 per cent; lymphocytes are relatively unaffected, showing a significant reduction in only one case; monocytes vary from "not significant"

CHART II

GROUP B. Leucopenia following intravenous injection of hemolyzed auto-blood



to 65 per cent. The *average* changes for the three patients are as follows: (1) total leucocytes 25 per cent reduction, (2) polymorphonuclear cells 30 per cent reduction, (3) lymphocytes 13 per cent reduction, and (4) monocytes 40 per cent reduction. Changes in eosinophiles and basophiles are so slight that they have not been recorded.

So much for the individual cases. Somewhat more interesting is a study of each group as a whole. To this end the reader's attention is directed to Charts I and II which are composite graphs of Groups A and B, respectively. These graphs do not necessarily reflect the events in any one case, but rather serve to indicate the general trend that a group may be expected to follow when subjected to the conditions of these experiments.

Group A

1. *Total Leucocytes.*—Within the first hour after injection, the white count develops a slight increase of 7 per cent above the basal average, then drops during the second hour to nearly the same level below the base line. Another peak is reached during the fourth hour with an increase of 25 per cent above normal; this is again followed by a depression. Then there is another gradual ascent until at the eighth hour the highest peak is reached, 40 per cent above the base line. Ten hours after the injection the count has fallen considerably, but still remains a little above the basal level.

2. *Polymorphonuclear Leucocytes.*—The pattern of this curve follows that of the above very closely, although the quantitative changes are somewhat greater. As in the first curve, the highest peak is reached in the eighth hour, this time rocketing 44 per cent above the basal level. The qualitative resemblance in the patterns of these two curves is in itself a hint that the changes in the hemogram are due primarily to the lability of the polymorphonuclear cells more than any other leucocytic constituent. This fact is corroborated by the numerical data in Table II.

3. *Lymphocytes.*—Here the pattern of the graph changes considerably. During the first two hours there is a progressive decrease to 30 per cent below the basal average. Not until the third hour does the count begin to rise, but then it ascends until at the fourth hour it has reached a peak of 23 per cent. This is followed by a gradual depression, then another elevation until it reaches a second peak, 27 per cent above the base line in the ninth hour. Thus, the lymphocyte curve lags at least an hour behind the polymorphonuclear cell curve at every point, again demonstrating the more sensitive lability of the latter type of cells.

4. *Monocytes.*—The fluctuations in these cells are much more irregular and inconstant than the others, and are therefore more difficult to describe. Just as in the case of the other cells, the highest peak occurs late, in this case ascending to 120 per cent above the normal average in the eighth hour.

Group B

1. *Total Leucocytes.*—Group B differs from Group A not only in direction but also in intensity; i.e., it develops leucopenia following autohemotherapy, and this leucopenia is quantitatively greater than the leucocytosis of Group A (see Table II).

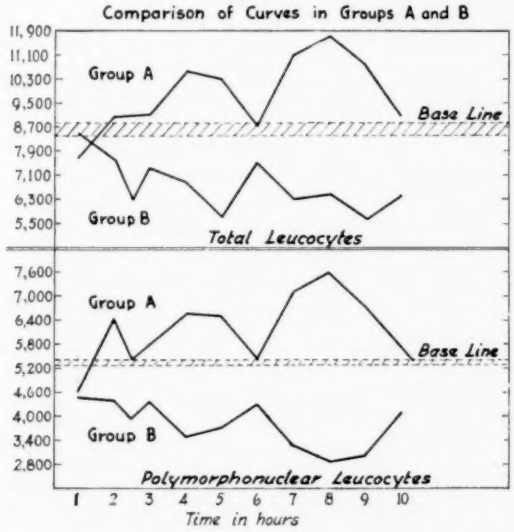
The initial reaction following injection is a progressive diminution in the white cell count until a point is reached 30 per cent below the base line during the second hour. Thereafter, just as in Group A, there are a series of peaks and depressions, never, however, rising above the base line, and finally reaching a low point of 36 per cent during the ninth hour. In fact, the *reaction is diametrically opposite to that of Group A.* Although at first glance nothing could seem more remote, closer examination reveals the peculiar fact that the patterns of this and the polymorphonuclear cell curves are almost mirror images of the same respective curves in Group A (see Chart III).

2. *Polymorphonuclear Leucocytes.*—Again the initial reaction is a drop, followed by the usual succession of peaks and valleys, the latter descending to lower levels each time and finally reaching the lowest point, 46 per cent below the basal level in the ninth hour. Above has been mentioned the curious fact that this curve is almost the direct reverse of the polymorphonuclear cell curve in Group A. If the reader will superimpose Chart I upon Chart II in such a manner that the base lines of the "poly" curves coincide and the corresponding peaks and depressions point in the same direction, the amazing similarity in the patterns of the two curves will be strikingly demonstrated (see also Chart III).

3. *Lymphocytes.*—Here also the initial reaction is a drop in the second hour, followed by a series of rises and falls. The greatest drop, in this case 47 per cent, occurs in the fifth hour.

CHART III

Comparison of Curves in Groups A and B



4. *Monocytes.*—Contrary to the three previous graphs, the initial reaction here is a sharp ascent to a peak 80 per cent above the basal level at the end of the first hour. Here also we see the characteristic peaks and depressions, the lowest point being reached in the sixth hour.

The tenacity of the leucopenia throughout Group B is evidenced by the fact that none of the curves reach the control base line even ten hours after the injection.

DISCUSSION

In our hands the intravenous injection of relatively large amounts of hemolyzed autoblood has been singularly free of discomfort, pain, or any other apparent ill effects. Even the feeble patients, R. B. and S. E., whom we regarded with considerable trepidation, fared no worse than their hardier brethren. Although we did not exceed 50 c.c. of blood or a total of 130 c.c. of hemolyzed solution in this series, we have reason to believe that larger amounts may be used without harm. Attention is called to the safety of the procedure and the

simplicity of technique not only for its therapeutic but also for its experimental implications; thus, the clinician and the hematologist will recognize in this medium an interesting means of studying such morbid conditions as hemolytic icterus, blood-destroying diseases like malaria, the mechanism of leucopenia and leucocytosis, etc.

A few facts already touched upon may require further comment in our discussion. There is, for example, the question of the use of citrated versus whole blood. Descarpentries[1, 2] emphatically denounces the use of anticoagulants, claiming that any substance that disturbs the physiologic functions of the blood to the point of preventing coagulation, must equally disturb other functions whose aim it is to defend the organism against infection. It would seem, however, that his objection is more hypothetical than real. The lysis of blood by distilled water in itself produces a profound alteration in physiologic functions, and it is difficult to conceive how the addition of a relatively innocuous anticoagulant can have any more deleterious effect. Besides, there is an important advantage in using an anticoagulant, since the process of hemolysis is hastened thereby, and the technique greatly simplified.

Another subject for discussion deals with the question of prolongation of hemolysis in the blood stream. Our experiments have indicated that, at least in vitro, when more blood is added to a hemolytic system in equilibrium, further hemolysis does not occur. When, as in our procedures, we employ a small excess of distilled water, i.e., a ratio of 3:2, it is possible that very slight additional hemolysis occurs at the moment of injection, but there is no evidence to indicate that it is prolonged, and for practical purposes it is so insignificant that it may be ignored.

So much for the details of technique. Thus far we have been dealing with self-evident facts that require little elaboration. We come now to the second phase of our work, namely, the leucopoietic response—a phase that is easier to describe than to interpret. In fact, any attempt to arrive at a final interpretation at this time could only result in a pedantic flight into the realms of abstract speculation far beyond our immediate horizon. Therefore, until we have collected more cases, we will confine ourselves in this report to a simple presentation of facts, at the same time raising such questions and making such suggestions as may seem pertinent to the material at hand.

The first and most important question that demands consideration is this: Why does one group of individuals develop leucocytosis and another leucopenia following the intravenous injection of hemolyzed autoblood? In Table I are listed a few of the factors that might conceivably influence the type of reaction; but careful examination of these factors throws little light upon the problem. Thus, the presence of complicating disease, the age of the patient, and the duration of syphilis show a similar distribution throughout both groups. We wondered if repeated bouts of fever might lessen the hematopoietic response. We found, however, that patients R. B. and S. E., although receiving no fever at all, fell into two different groups; and that C. C., in spite of eighty hours of fever to his credit, developed a leucocytosis.

Does the amount of blood that is injected have any influence upon the type of reaction? W. S. and S. E., who received the largest amounts of blood, both developed leucopenia; but, on the other hand, C. K., with only 30 c.c. of blood also developed leucopenia, whereas R. B. with 40 c.c. of blood exhibited the opposite reaction. The relationship is therefore not clear as yet, although we hope that further investigation will solve this important problem.

Although at first glance nothing would seem more remote than the possibility of a connecting link between the opposing reactions of Groups A and B, careful analysis of the data and the three charts will reveal many points of similarity between them. A striking characteristic in each case is the irregularity of all the curves. The elevation or depression does not proceed evenly upward or downward, but rather by a series of irregular, tidelike fluctuations, until the peak of leucocytosis or the trough of leucopenia is reached. Another important similarity is the late period in which the maximum leucocytosis or leucopenia develops. This fact emphasizes the necessity of making frequently repeated observations over long periods of time. Again, in Group A the leucocytosis is due primarily to an increase in neutrophilic polymorphonuclear leucocytes, the lymphocytes being at first depressed and increasing relatively later. In Group B the leucopenia is also chiefly polymorphonuclear in nature, the lymphocytes being relatively unaffected. We cannot resist the opportunity, at this point, to call attention to the many points of similarity between this type of neutrophilic leucopenia and the leucopenia of malaria, and to suggest that blood destruction is in both cases the common factor which may be responsible for that reaction. We hope to develop this conception more fully in our forthcoming report on autohemopyretotherapy.

We have spoken of the various characteristics that Groups A and B possess either separately or in common. But the one that has piqued our curiosity more than any other still remains to be discussed. We refer to the mirror-image relationship between the total leucocyte and, even more strikingly, between the polymorphonuclear curves of the two groups. In our opinion, this phenomenon, more than any other, establishes definitely the specific nature of our results.

We realize that seven patients do not form a very imposing array from which one might be tempted to draw final conclusions; but, on the other hand, they have been so painstakingly investigated and have yielded such significant data that the subject seems to be worthy of considerable additional investigation. This we are planning to undertake. Meanwhile, it is hoped that this preliminary report, by calling attention to the many interesting problems involved, may lend added impetus to the experimental study, not only of the newly conceived autohemopyretotherapy, but also autohemotherapy in general. Finally, in closing our discussion, we would like to make this plea: Let those who are interested in elevating autohemotherapy to the rank that it merits devote attention not alone to its therapeutic manifestations, but also to the scientific mechanism underlying them; for one must depend primarily upon the experimental approach if it is desired to establish autohemotherapy upon a permanently sound foundation.

SUMMARY

1. A simple method is described for the intravenous injection of hemolyzed, citrated, autogenous blood. The use of this technique makes possible the injection of relatively large amounts of blood, without ill effects.

2. According to the nature of the leucocytic reaction following the injection, individuals are found to fall into two groups: Group A exhibits leucocytosis; Group B, leucopenia. Separate characteristics and points of similarity between the two groups are pointed out, the most striking being the fact that the curves of the total leucocytes and polymorphonuclear cells of each group, although opposed in direction, are, nevertheless, mirror images of each other. These phenomena are taken to indicate the specific nature of the results obtained.

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CHANGES IN ERYTHROCYTE FRAGILITY DUE TO PHYSICAL EXERCISE AND VARIATION OF BODY TEMPERATURE*

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IN 1921 Hastings[1] showed that the resistance of erythrocytes to laking by hypotonic salt solutions is increased in dogs immediately after a period of treadmill running. Lieberman and Acél[2] reported similar findings in rabbits, guinea pigs, and men, with the exception that they observed a decreased erythrocyte resistance for ten minutes immediately after the exercise period.

Thörner[3] reported an increase of erythrocyte osmotic resistance in dogs as a *chronic* effect of daily physical training by treadmill running. Davis and Brewer[4] reported a decreased erythrocyte resistance in one dog trained by treadmill running, and an increased erythrocyte osmotic resistance in two dogs trained by daily swimming in water at 30° C. These workers suggested that the body temperature during exercise may be an important determining factor.

One purpose of this investigation was to obtain more data upon the chronic effect of treadmill running on cell resistance in dogs. Another objective was to

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investigate a possible relationship between body temperature and erythrocyte osmotic resistance.

PROCEDURE

Five dogs were confined in a large cage and placed on a constant diet of Purina dog food for two weeks before control determinations on the blood were made. Erythrocyte fragility determinations were then made on each dog by Hasting's method[1] at various times over periods of from one to two and one-half weeks. Erythrocyte numbers and hemoglobin percentages (Sahli) were also determined. The blood was drawn from the saphenous vein, after the dog had

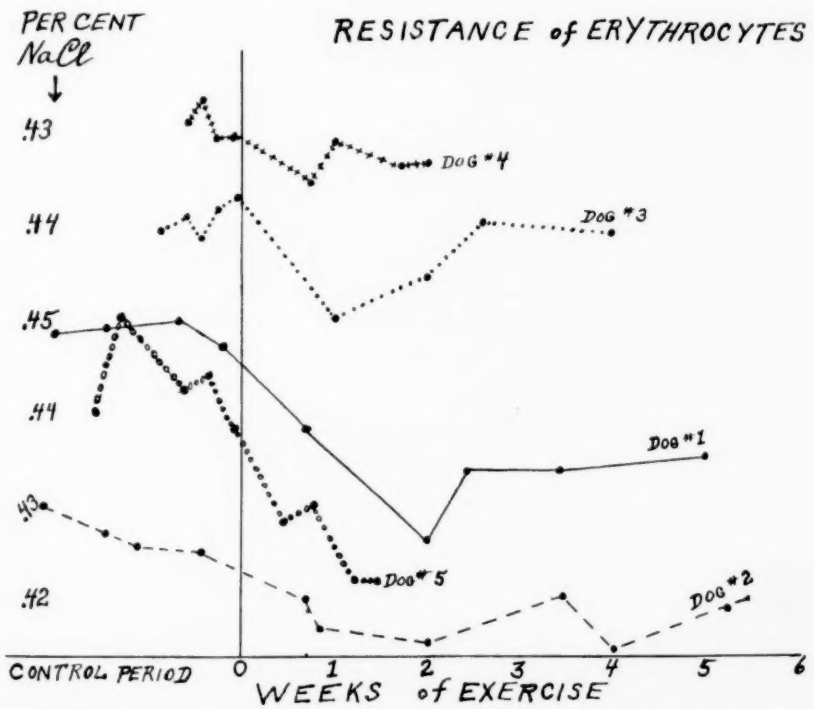

Fig. 1.—The chronic effect of daily treadmill running upon the osmotic resistance of erythrocytes in five dogs. The resistance is expressed as the percentage of sodium chloride solution that causes 50 per cent hemolysis.

rested quietly (blindfolded) on a table for twenty minutes, and was also in a basal state with respect to previous exercise and food.

The daily exercise during the training period consisted of running a distance of 5 miles in one hour on a treadmill inclined at a 25 per cent grade. A few minutes of rest was always allowed the dogs after the first half-hour of running.

In later studies designed to investigate the influence of body temperature upon red cell fragility, dogs were immersed in a tank of water for a period of thirty minutes. The water in the tank was maintained at 43° C. in experiments intended to raise the body temperature, and at 22° C. when it was desired to reduce the rectal temperature.

RESULTS

Chronic Results.—In all five dogs, the erythrocytes became less resistant osmotically during the first week of treadmill running. This result is not in agreement with the work of Thörner[3] (Fig. 1). The resistance is expressed in terms of the concentration of sodium chloride which caused 50 per cent hemolysis in one hour. Dog No. 4 died of pneumonia after losing weight rapidly, but all of the other animals appeared to be exceedingly healthy and lost no weight throughout the experimental periods.

During the first week of regular exercise, the hemoglobin per unit volume of blood was reduced in all dogs by 10 to 18 per cent of the preexercise normal value, and the erythrocyte numbers were reduced correspondingly. After 3.5 weeks of training, the erythrocyte numbers of Dogs 1 and 2 exceeded the preexercise normal value by 20 and 14 per cent, respectively. These findings confirm previous work by Davis and Brewer.[4]

It was suspected that the apparent fragility of erythrocytes might be related to changes in the osmotic pressure of the blood serum due to regular daily exercise. Therefore, the freezing point depression of the serum was determined occasionally in two of the dogs on blood from the same samples which furnished material for fragility tests. These determinations were made with a Heidenhain thermometer on 5 c.c. samples of blood serum. No correlation was found in these experiments between the erythrocyte osmotic resistance and the serum osmotic pressure.

TABLE I

AVERAGE CHANGES IN THE BLOOD OF TWO DOGS IMMEDIATELY AFTER TREADMILL EXERCISE

	DOG NO. 3		DOG NO. 2	
	BEFORE EXERCISE	AFTER 20 MINUTES RUN	BEFORE EXERCISE	AFTER 30 MINUTES RUN
Erythrocyte number	4,800,000	5,570,000	5,770,000	7,440,000
Hemoglobin per cent (Sahli)	67	79	85	100
Hematocrit cell volume	27 per cent	32.5 per cent	34 per cent	43.5 per cent
Body temperature (Fahrenheit)	101.3°	105.4°	100.8°	105.1°
Serum osmotic pressure (△)	0.526	0.551	0.547	0.557
R.B.C. Fragility as per cent NaCl for 50 per cent laking	0.440	0.414	0.471	0.453

Results Immediately After Exercise.—Table I is presented to show changes in body temperature, erythrocyte fragility, and serum osmotic pressure that were observed immediately after exercise. All values given for each dog are the average of three experiments. In all experiments all of the values for each determination changed in the same direction, except in one experiment on Dog No. 2 in which the serum osmotic pressure was observed to be diminished after exercise. The increased osmotic resistance reported here confirms the work of Hastings[1] on the dog, and the older work of Manca[5] (1896), cited by Hastings,[1] for human beings. Hastings found an increased chloride content of the plasma, and a decreased chloride content of the erythrocytes after exercise, and suggested the possibility that the osmotic pressure of the cell contents may be diminished.

TABLE II

EFFECT OF HOT AND COLD BATHS ON THE DOG

	HOT BATH IN WATER AT 43° C. FOR 30 MINUTES			COLD BATH IN WATER AT 23° C. FOR 30 MINUTES		
	BEFORE	IMMEDIATELY AFTER	24 HOURS AFTER	BEFORE	IMMEDIATELY AFTER	24 HOURS AFTER
Erythrocyte number	5,190,000	6,310,000	----	4,930,000	6,920,000	4,860,000
Hemoglobin per cent (Sahli)	72	95	----	68	91	75
Hematocrit	27 per cent	34 per cent	----	32 per cent	40 per cent	35 per cent
Body temperature (rectal)	101.9°	104.4°	101.8°	101.3°	99.6°	101.0°
R.B.C. Fragility as per cent NaCl causing 50 per cent hemolysis	0.427	0.417	0.437	0.444	0.464	0.430

Hot and Cold Baths.—Table II shows typical experiments in which dogs were given hot and cold baths of thirty minutes' duration. Immediately after a hot bath, the osmotic resistance of red cells is increased, but twenty-four hours later it is decreased to a value below the normal. The increase of rectal temperature observed immediately after the bath was about 2.5° F. This was not so great as that observed after exercise, but the increase in erythrocyte resistance was also correspondingly less. Cold baths caused just the opposite changes in resistance, namely, a decrease immediately after, and an increase above normal twenty-four hours after the bath. The body temperature was lowered by about 1.7° F. by the cold baths. Practically no exercise was involved in these baths.

No correlation was found to exist between occasionally determined serum osmotic pressure and erythrocyte fragility after the baths.

DISCUSSION

I believe that the subnormal cell resistance observed twenty-four hours after a hot bath is caused by the same factors which produce a diminution of resistance during a period of physical training by treadmill running, and that the elevated body temperature during running or hot bathing is primarily responsible for the observed changes in red blood cell resistance. Similarly cold baths seem to affect the cell resistance to laking in the same manner as exercise by swimming in cool water, as regards the chronic or delayed effect previously reported by Davis and Brewer.[4]

Rice and Steinhaus[6] have shown that treadmill running produces in dogs an increase in the pH of the blood, and that this is due to overheating, overventilation, and blowing off of carbon dioxide by the lungs. Their dogs were run for about one-half an hour at a speed of 5 miles per hour on a treadmill inclined at 25 per cent grade. These exercise conditions are almost identical with those which prevailed in my experiments given in Table I. Since I did not attempt to determine the pH of the blood, I have not precluded the possibility that this is a determining factor in red corpuscle resistance changes due to physical exercise.

CONCLUSIONS

Daily treadmill running produces in dogs a decrease in the (resting) resistance of erythrocytes to laking by hypotonic salt solutions during the first week of training. The erythrocyte osmotic resistance, as determined *immediately* after running exercise, is increased.

The body temperature during exercise is probably a primary factor in causing this chronic decrease due to daily running exercise as well as the previously reported increased resistance produced by daily swimming in water at 25° to 30° C. Hot baths caused the same changes of erythrocyte fragility as treadmill running, while cold baths produced the same chronic change as swimming.

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THE EXCRETION OF SULFANILAMIDE IN PERSPIRATION*

WALTER J. HIGHMAN, JR., CH.E., CHICAGO, ILL.

THE measurement of the sulfanilamide excreted in urine has been proposed by Marshall[1] as a method by which the dosage of prontylin might be measured and by which the efficacy of that dosage might be estimated. The low excretion of this drug in the urine of one of our patients during an extreme hot spell led us to extract his clothing and to test the extract qualitatively for sulfanilamide. We found that appreciable amounts of this compound were recovered from the perspiration contained in the clothes. The clothes of five subsequent patients on this medication were extracted. Prontylin was recovered in appreciable quantities in each case. We feel, therefore, that in hot weather, the excretion of para-amino-benzenesulfonamide in perspiration may lead to its lowered excretion in the urine.

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DEGENERATIVE LEUCOCYTIC TRANSFORMATIONS ASSOCIATED WITH AGING*

Differential Identification of Monocytes

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THE life span of human white blood cells in the peripheral blood stream varies from a few hours to approximately four days.[1-4] White blood cells in sealed (vaseline) cover slip preparations have a similar life span. Such preparations with supravital stains[5] offer a means for studying viable cells. Together with other factors, the viability of white blood cells is determined by the cell motility, the movement of granules and vacuoles within the cytoplasm, the size of the vacuoles, the Brownian motion, and changes in the staining characteristics of these intracellular structures. In the original technique of Sabin both neutral red and Janus green are employed for staining purposes. The former is only slightly toxic to cells,[6-8] whereas the latter does not permit the cells to survive more than three to four hours and, therefore, is not satisfactory for prolonged studies. In view of this fact, we employed the Sabin technique with the exclusion of Janus green to study the life span of white blood cells at room temperature.

Of the many changes which occur during the life span of a white blood cell in vitro, those associated with the vacuoles are most interesting; the process of autolysis being the final stage. Vacuoles take up the neutral red stain and thereby become readily visible. It is believed by some that vacuoles are cytoplasmic invaginations in which digestive processes take place, and that the enzymes produced for these digestive processes absorb the neutral red.[9, 10] White blood cells, when grown on tissue culture media, develop vacuoles with age.[11] We have observed that this also occurs in the neutral red supravital preparations and that these vacuolar changes are chiefly responsible for the morphologic alterations of the cells during cellular degeneration.

The majority of white blood cells of human peripheral blood develop vacuoles in these supravital preparations. Among other changes we have observed specific vacuolar alterations in the monocytes. These are quite uniform and constant in their development. During the first ten to twenty minutes of staining the monocytic vacuoles are small (size of neutrophilic granules), of salmon pink color, and often arranged in a rosette. For the next two to three hours they enlarge gradually until a nearly uniform ovoid shape of about one micron

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in diameter is seen. At this stage we find the cytoplasm of the monocyte filled with uniformly stained vacuoles; the cell itself becomes nonmobile and assumes a spheroid shape. Such monocytes resemble macrophages because of the numerous vacuoles which are common to the latter. The development of the vacuoles during the first two to three hours may be due to some process associated with

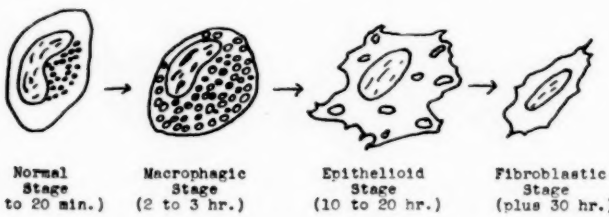

Fig. 1.—Diagrammatic presentation of sequence of degenerative changes observed in neutral red supravital preparations of monocytes. See text.

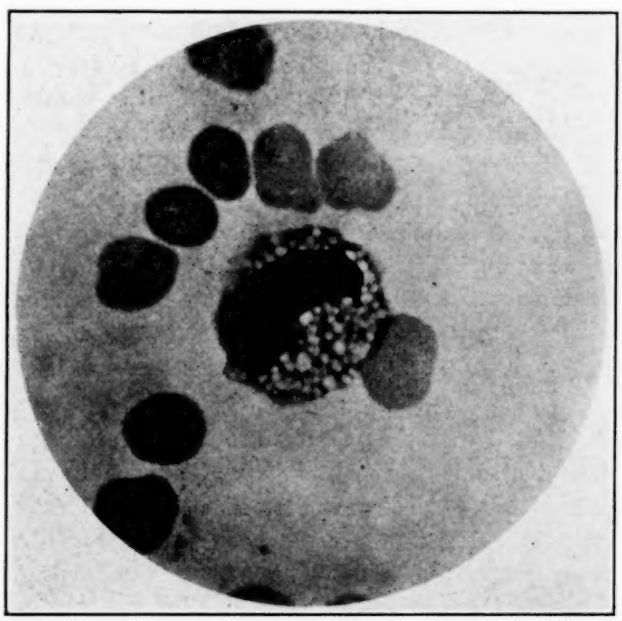

Fig. 2.—The "macrophagic" stage of a normal human monocyte from a three-hour-old cover slip preparation after fixation and staining by the Jenner-Giemsa technique (×2000). See text.

starvation, since Koehring observed that paramecia grown on rich media developed few vacuoles, whereas on nutritionally inadequate media vacuolar development was marked.

After this "macrophagic" stage is maintained for a number of hours (five to twenty-four hours), the monocytes begin to degenerate and the vacuoles continue to expand and subsequently rupture and lose their red-stained contents. Some of the monocytes become a degenerated colorless mass within forty-eight hours while others partially maintain their cytoplasmic continuity and enlarge, assuming an appearance similar to epithelioid cells. Later, when the cytoplasm

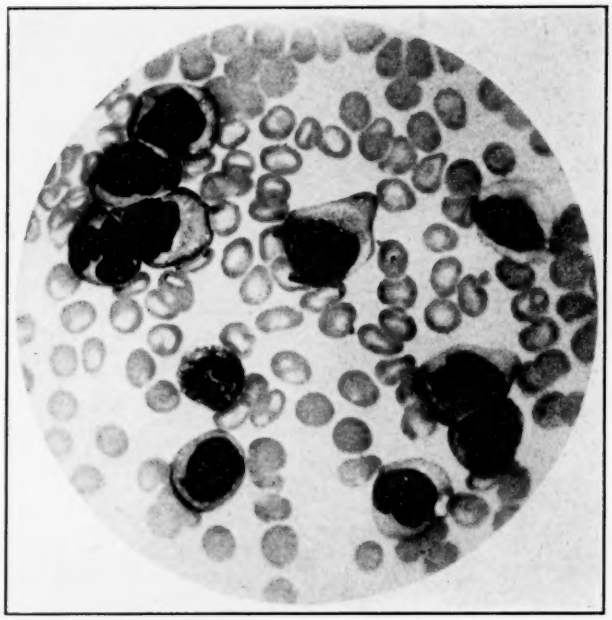

Fig. 3*A*. (×1000)

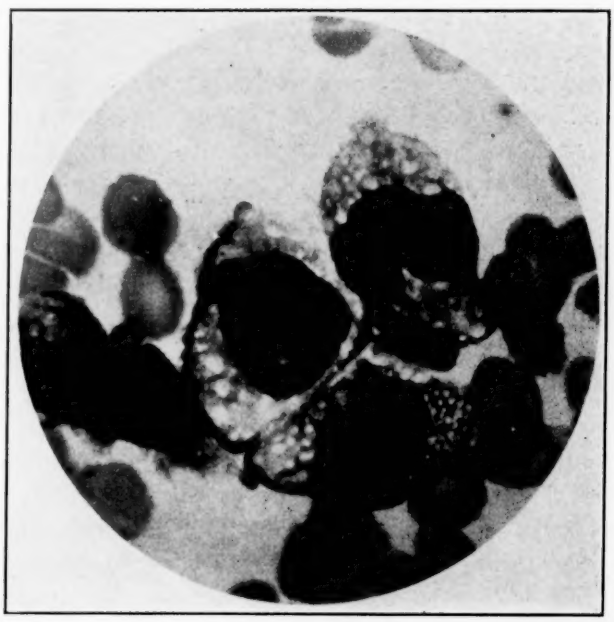

Fig. 3*B*. (×2000)

Fig. 3.—Blood from a case of monocytoid myeloblastic leucemia, illustrating the effect of aging in a neutral red supravital preparation; *A*, before the preparation and *B*, five hours after preparation, showing the "macrophagic" state of vacuolar degeneration.

becomes frayed, with all of the vacuoles ruptured, some cells assume an appearance similar to that of fibroblasts. Apparently only the very viable cells go through all of the morphologic stages described above. The sequence of events is illustrated diagrammatically in Fig. 1.

It is possible to obtain permanent records of any of these stages by gently removing the cover slip and then fixing and staining the blood smear. This is illustrated by Fig. 2, which demonstrates a Jenner-Giemsa stained cell at the three-hour stage when vacuolated monocytes resemble macrophages in appearance.

The above-mentioned observations become of practical interest when it is difficult to differentiate between monocytes and promyelocytes. The development of numerous vacuoles in the monocytes in a neutral red supravital preparation offers a definite criterion for the identification of these cells. However, if the cells under question do not show uniform vacuolization within three hours, they are in all probability myelocytes.

As we have already stated, vacuolization is common to most white cells on aging, but the extent of this process is not so characteristic in other cells as it is for the monocytes. The fact that different white cells develop numerous vacuoles and thereby approach macrophages has given rise to the concept that macrophages may originate from other cells.

Awrorow and Timofejewskij[12] concluded that myeloblasts and lymphocytes were changed to macrophages when leucemic blood was cultured. We have observed similar changes with the neutral red supravital technique (Fig. 3). From the morphologic point of view it is difficult to distinguish a true macrophage from the "macrophagic" stage of various degenerated cells. However, whereas the true macrophage is actively phagocytic for debris and fragmented red cells, the "macrophagic" cells do not exhibit a similar functional activity.

In addition to the above it is believed by many that lymphocytes develop into macrophages,[13-15] that reticulum cells develop into macrophages,[16] that monocytes are transformed into macrophages and subsequently into epithelial cells,[17-20] and that both myeloid and lymphatic leucemia blood cells[21] change to vacuolated "macrophagic" cells. With the neutral red supravital technique the development of the "macrophagic" stages in lymphocytes, reticulum cells, monocytes, lymphoblasts, and myeloblasts can be observed.

Although polymorphonuclear leucocytes do not usually undergo appreciable changes in neutral red supravital preparations, it is possible to observe the "macrophagic" stage even in these cells. This may be exemplified by the changes observed in the polymorphonuclear leucocytes in septic tissue sites (Fig. 4).

The fact that so many different types of cells assume a similar appearance during aging, but do not reveal the specific functional aspects of macrophages, is indicative of the possibility that these cells do not become true macrophages but are merely passing through one aspect of the degenerative process that we have described above.

It seems to us that if true macrophages can develop from monocytes, one should find many more macrophages in tuberculosis, leucopenic infectious monocytosis, undulant fever, Hodgkin's disease, etc., in which monocytes are abnormally increased. Furthermore, it might be expected that in such instances in which macrophages are found in increased numbers (malaria, kala azar,[22] subacute bacterial endocarditis, and syphilis[23]), an associated or proceeding monocytosis would be observed. Likewise, there is no increase in the number of macrophages following the monocytosis of the blood[24] of human subjects subjected to tuberculin injections.

These considerations lend emphasis to our observations that the development of cells resembling macrophages is not indicative of a functional transformation but is merely a degenerative phenomena associated with aging of the

Fig. 4.—Polymorphonuclears and monocytes obtained from the pus of a furuncle. These cells, through vacuolated degeneration, have developed a macrophage-like appearance.

cells irrespective of whether this process occurs in vivo as a development of so-called macrophages in pleural, abdominal, and other cavities, or in vitro as in the neutral red supravital technique.

SUMMARY AND CONCLUSIONS

1. Various stages of degeneration of white blood cells have been observed with the neutral red supravital technique (Fig. 1).

2. Monocytes can be identified by characteristic vacuolar changes (Fig. 2).

3. The development of a "macrophagic" stage by various white cells during aging indicates that this is a degenerative process and not a transformation of one physiologic type to another.

The author wishes to express his appreciation to Dr. N. Rosenthal for his interest and aid.

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LEUCOCYTIC VARIABILITY AND ITS RELATION TO METEOROLOGIC ALTERATIONS*

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IN RECENT years many papers have appeared discussing leucocytic variability and the various factors that influence it. It is the purpose of this paper to discuss the influence of the meteorologic alterations on the leucocyte count.

In reviewing the literature, our attention was first called by Alvarez[1] to a paper on Filariasis which he wrote in which he noted changes in the eosinophile-lymphocyte level and variations in the neutrophile count associated with the cyclic changes occurring with the moon in Porto Rico. In a later personal communication he observed an increase in the leucocyte count and the polymorphonuclears associated with the period of low barometric pressure during the cyclone of St. Nicholas, which passed over Porto Rico on September 10, 11, and 12, 1931. Stetson (1927)[2] observed a seasonal variation in the polymorphonuclear cells, with the highest average percentage in June and the lowest average percentage in December. On the other hand, the highest average percentage of lymphocytes was in December. He also found day to day fluctuations in the white blood count in which each type of cell participated. These fluctuations were beyond the limits, dependant upon technical or mathematical errors. Harvey and Hamilton (1934)[3] also observed daily variations in the blood count. In one allergic individual these fluctuations were much greater then in the normal. Zirm and Bauermeister (1933)[4] and others have also observed abrupt changes. Shaw (1934)[5] observed a case in which he made leucocyte counts for the period of one year. These observations were made in Cairo, Egypt. He found a persistent left-handed deflection, and no seasonal variations. He observed that the range of variation in the year is the same as in one person in twenty-four hours but less than in a population. Kennedy and Mackay (1935)[6] observed that the polynuclear count of healthy British airmen in Iraq was not significantly different from the indigenous population, and both groups were definitely deviated to the left as compared with health standards in Britain. Also pathologic cases and normal rabbit counts were more left-handed than in Britain. The authors suggested that the most probable causative factor was climatic.

In the summer of 1932,[7] we became interested in studying these daily fluctuations in the leucocyte count. We made daily counts under basal conditions at the same time every morning on four normal individuals. The pipettes and counting chamber were certified by the U. S. Bureau of Standards, and the same pipette was used throughout the study for each individual.

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Of our four normals, subject No. 1 was of a pyknic habitus; No. 2, a mixed type; No. 3, a leptosome; and No. 4, a leptosome of somewhat heavier proportions.

TABLE I

PATIENT NO.	AVERAGE COUNTS	
	SUMMER	WINTER
1	9,350	10,600
2	5,700	
3	7,790	8,460
4	4,590	

We observed that the average level of the count and the degree of fluctuation varied with each individual (see Table I). Subject 1, with the pyknic habitus, showed the highest level, while No. 4, a leptosome who was strongly sympathicotonic, showed the lowest average level; and Nos. 2 and 3 occupied intermediate positions. In comparing the summer levels (July and August) with the winter levels (December and January) we found that the average counts in winter were higher than those of the summer, while the degree of fluctuation was greater in the summer than in the winter. The individual with the pyknic habitus not only had the highest level, but also showed the greatest degree of fluctuations, while the strongly sympathicotonic individual not only showed the lowest level, but also showed the lowest degree of fluctuations.

Variations in levels with constitutional differences have been previously observed by Moewes[8] and Isaacs,[9] while fluctuations of the counts around various levels have been observed by Doan and Zerfas,[10] Smith and McDowell,[11] Jackson and Stovall,[12] Shaw,[13] and Harvey and Hamilton.[3]

The average levels of the differential counts may be observed in Table II. The neutrophile levels are slightly lower in winter in subject 1 and almost the same level in subject 3; however, both basophile and eosinophile levels are noticeably elevated in both in the winter.

TABLE II

	SUMMER				WINTER	
	1	2	3	4	1	3
Neutrophiles	66	67	66	55	61	64
Lymphocytes	31	30	32	46		
Eosinophiles	0.86	0.76	0.74	0.35	2.3	3.5
Basophiles	0.06		0.21	0.19	0.27	0.65
Monocytes	2.2	1.82	1.82	2.06		

Of particular interest are the daily leucocyte counts made during the summer and winter and the correlation of the counts with meteorological alterations.[14] Chart 1 is presented, summarizing the results in subjects 1 and 3.

At the top of the chart is the barograph. The line at the top of the graph represents the wind velocity; the blocks of black and white, the percentage of sunshine and cloudiness; the heavy line, the barometric pressure; the dotted line, the temperature; and the vertical lines, the degree of weather instability. The associated peaks with the meteorologic episodes are lettered from A to Y.

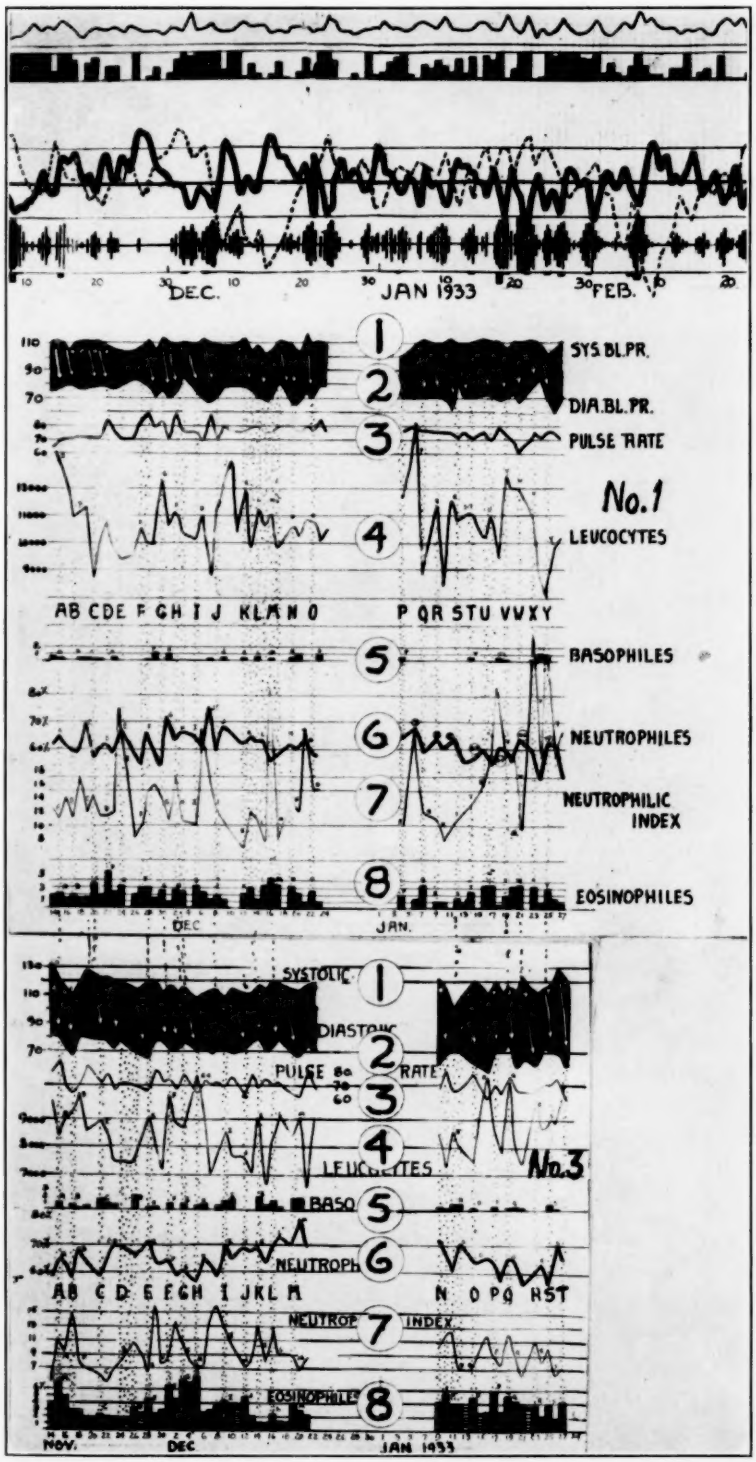
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Chart 1.

In viewing the graphs we note that following the polar infall (rising barometric pressure and falling temperature) of November 15 (episode 1) there is a slight rise in the eosinophiles, a fall in the nuclear index[15] (more young forms), a rise in the neutrophiles and total leucocyte count, and a fall in the diastolic pressure. On November 21 (episode 4) there is a marked polar infall. This is associated (response D) with a rise in systolic blood pressure, followed by a fall in the diastolic pressure, a rise in the total leucocyte count and eosinophiles, and a fall in the nuclear index. A similar sequence of events of varying degrees is found with most of the meteorologic episodes.

In January frequent polar infalls occur with marked changes. In general, the more pronounced the meteorological, the more pronounced the peaks in the leucocyte picture. The degree and promptness of the response varied with the individual.

The meteorologic alterations have been shown for the most part to be associated with pressor episodes. Following these, there then occurs a vascular dilatation with a fall in diastolic pressure which is associated with an increase in the eosinophiles, the appearance of more young forms, and increase in the neutrophiles and in the total leucocyte count. The fluctuations may in part be explained by the emptying of the reservoirs of cells, such as the spleen, liver, and lungs.[16-18] Meyer, Seevers, and Beatty[18] observed a temporary leucocytosis in rate following a reduction in barometric pressure. Elias and Kaunitz[19] had previously observed that reduced atmospheric pressure producing an anoxemia resulted in a leucopoiesis. Alterations in the splanchno-peripheral balance,[20, 21] with splanchnic dilatation and peripheral vasoconstriction and vice versa, probably play a rôle.

Besides these factors of release of leucocytes from the bone marrow, redistribution of cells, and alterations of splanchno-peripheral balance, the stimulation of the bone marrow by the relative anoxemia resulting from the pressor episodes must be considered.[14] Elias and Kaunitz[19] have demonstrated the influence of anoxemia causing a leucopoiesis, and an associated hunger increased the response while feeding carbohydrates decreased the level evidently by counteracting the acidosis. Murakami[22] also observed the influence of acidosis on increasing the polymorphonuclear leucocytes. Bier,[23] Freeman,[24] and others have observed the influence of anoxemia and the increase in tissue metabolites in stimulating flow of blood. In our group of normals at the onset of a polar infall, there is a relative leucopenia with a diminished number of neutrophiles, as well as an increase in systolic pressure; however, at the crest of the cold front and during the development of a tropical wave, the systolic pressure is generally lowered and the diastolic pressure falls (anoxemic phase[14]) and an increase in the number of eosinophiles and young forms, an increased frequency of appearance of basophiles, as well as an increase in the total count prevails. During the winter, with the frequent passages of the cyclonic interfaces, with pronounced swings in cold and warm weather, a greater stimulation is evident in the higher leucocytic level, the increase in eosinophiles, and the higher levels of fluctuation.

The leucocytic levels and the degrees of fluctuations are conditioned by the constitutional make-up of the individual.

A series of daily studies on groups of patients were carried out at various periods from 1933 to the present day. Because the volume of material would be too cumbersome to present, we have confined ourselves to the daily records of five of the patients.

In Chart 2 may be seen the record of patients J and G which was made during June and July of 1934. The record of J is placed beneath the barograph, while the record of G is at the bottom of the chart.

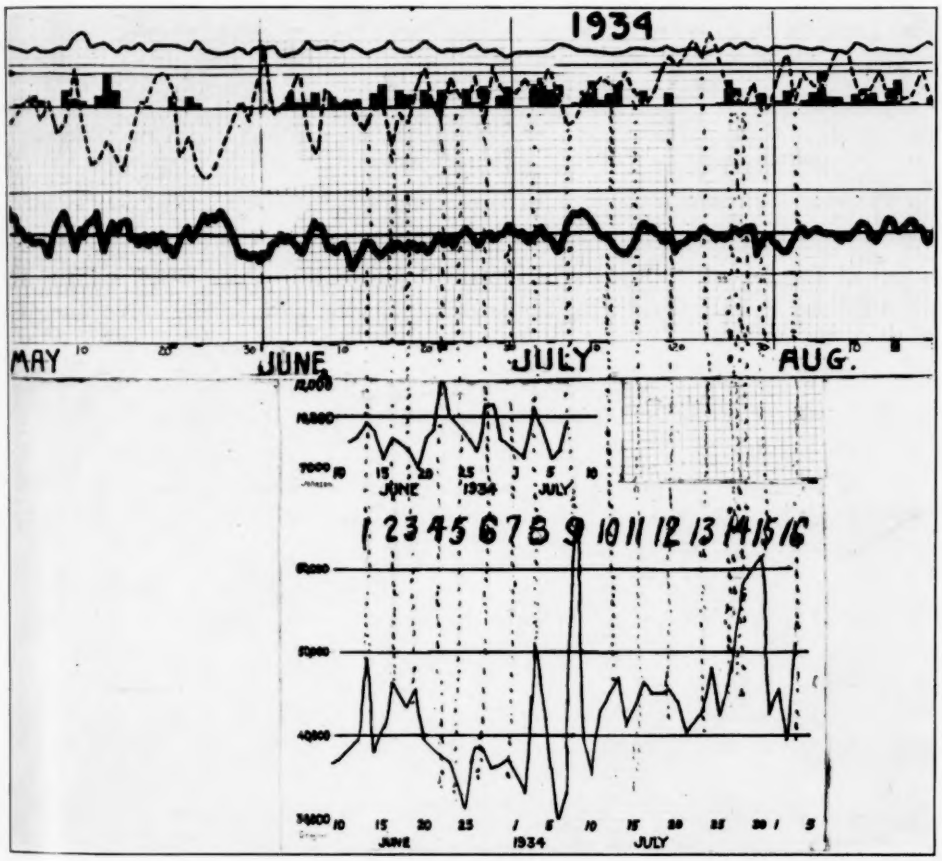

Chart 2.

In looking over the chart of J (aleucemic lymphadenosis) we observe that nine polar episodes occurred and with seven of these peaks in the leucocyte count are associated (77 per cent). The peaks are slender with reversal taking place rapidly after the rise to reach a rather uniform base line.

On the other hand, the record of G (myelogenous leucemia) shows wide fluctuations. With fourteen of the sixteen polar episodes that occurred, leucocytic peaks are found (87 per cent). In this case we see the pronounced redistribution of cells as well as stimulation of bone marrow which probably occurs

with the polar episodes. This is a different picture than is seen in the case of lymphatic leucemia (see patient Cr). In comparing the peaks of J and G, a synchronism which is very close is observed, although the characters of the peaks are different.

The six-month record of a patient (R) with chronic far-advanced pulmonary tuberculosis reveals the following:

For the most part the peaks in the total leucocyte count occur with peaks in the neutrophiles; at times they occur with lymphocytic increases. A day or two after a peak in the immature cells, a peak in the neutrophiles occurs. This rhythm in shifting to the right in this patient occurs regularly almost without exception. The peaks in the neutrophiles and total leucocytes are not alone the result of the increase in immature forms, but the redistribution of cells, hydration, and other factors play a rôle. The eosinophile level is low and the cells appear at irregular times. Swings in the total leucocyte count do not occur with as marked rapidity as are seen in many of the other cases.

During the autumn the general level of the total leucocytes is higher and the fluctuations are greater than during the winter.

The per cent of immature neutrophiles is greater in the autumn (average 17 per cent) than in the winter (average 12 per cent), while the fluctuations are greater in the first period.

During the autumn, the better response in the immature cells and increased level of leucocytes are probably due in a good measure to the greater activity of the bone marrow.

The record of another patient (H) with far-advanced pulmonary tuberculosis revealed a similar daily record to the previous case. Increases in the leucocytes are associated with most of the polar episodes (73 per cent).

In the daily study of a case of chronic lymphatic leucemia, we found that the swings in the leucocyte count are sharp and abrupt. It is very interesting to note that the majority of the polar infalls are associated with depressed levels in the leucocyte counts, while the peaks occur after the polar episodes. This, as has been pointed out, is quite the opposite of what transpires in the patient with myelogenous leucemia.

SUMMARY

Alterations in the meteorologic environment bring about comparable changes in the human subjects under our observation. In our normals a period of increased systolic and diastolic pressure (vascular spasm) is followed by an increase in eosinophiles, the appearance of more immature forms, a rise in the polymorphonuclear leucocytes and total white count. The level and the degree of fluctuations are in a measure conditioned by the constitutional habitus of the individual. Seasonal variations are observed.

In our group of patients variations in the leucocyte count occurred with the meteorologic episodes; the degree and character of the fluctuations are influenced to varying degrees by the pathologic condition of the patient. Summaries of the daily records of five of our group of patients are presented.

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FORMATION OF ABNORMAL BLOOD PIGMENT AS A COMPLICATION OF SULFANILAMIDE THERAPY*

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IN THE past few years chemotherapeutic measures against acute infections have become increasingly important. Among such, sulfanilamide is outstanding. That it produces cyanosis is recognized. Our experience with the causative agent presents several interesting features.

A mild degree of cyanosis is often seen to develop on administration of sulfanilamide and to persist without much change during the course of the therapy. Obvious cyanosis noted early in the treatment may noticeably diminish despite continuance of the drug, although its disappearance is conditioned by withdrawal of sulfanilamide. On the other hand, the cyanosis may become sufficiently intense to assume clinical importance. Four such cases are presented herewith, the causative agent being subjected to chemical scrutiny.

CASE 1.—Female, aged 33 years, with ulcerative colitis confirmed by sigmoidoscopy and stool examinations; on culture hemolytic and nonhemolytic streptococci predominated, negative repeatedly for *E. histolytica*, *B. dysenteriae*, and tubercle bacillus. Table I indicates the various therapeutic measures and their relationship to cyanosis.

CASE 2.—Male, 3 years of age, with bilateral cervical adenitis; blood culture positive for hemolytic streptococci, likewise pus evacuated from incised swelling on ankle; pneumococci obtained on incision of both eardrums. The relatively large dose of sulfanilamide required to produce cyanosis is shown in Table I together with other therapy employed.

CASE 3.—Male, 31 years of age, with sinusitis; culture of exudate from punctured antrum positive for hemolytic streptococci. Cyanosis and dyspnea appeared within eight hours of first dose of sulfanilamide (1.2 grams) and one hour after the second dose. Oxygen therapy did not relieve the cyanosis until the drug was discontinued. The cyanosis cleared completely with oxygen forty-four hours after the last dose of sulfanilamide. Other therapy is given in Table I.

CASE 4.—Male, aged 3½ years, with pneumonia with left empyema; thoracentesis yielded greenish pus containing hemolytic streptococci; excoriating serosanguineous nasal discharge on culture revealed *C. diphtheriae* (antitoxin administered); no further evidence of diphtheritic involvement. After 19.2 grams of sulfanilamide, cyanosis was evident. Coincidental respiratory embarrassment was greater than could be attributed to the pulmonary disease. An oxygen tent did not relieve the cyanosis. The course of treatment is given in Table I.

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TABLE I

DAYS	CASE 1.—ULCERATIVE COLITIS					CASE 2.—HEMOLYTIC STREPTOCOCCIC SEPSIS						CASE 3.—HEMOLYTIC STREPTOCOCCIC SINUSITIS						CASE 4.—HEMOLYTIC STREPTOCOCCIC EMPYEMA					
	ATROPINE SULFATE	SODIUM PHENOBARBITAL	BISMUTH SUBCARBONATE	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC	ACETYLSALICYLIC ACID	SULFANILAMIDE	PRONTOSIL	CODEINE SULFATE	CYANOSIS	SPECTROSCOPIC	ACETYLSALICYLIC ACID	ACETPHENETIDIN	CODEINE SULFATE	MORPHINE SULFATE	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC	PHENOBARBITAL	ACETYLSALICYLIC ACID	SULFANILAMIDE	CYANOSIS	SPECTROSCOPIC
1	0.001	0.29	32.0				0.3						1.5	0.48										
2	0.001	0.29	32.0				1.0						1.5	0.48										
3	0.001	0.29	32.0	0.3			0.6	0.2	0.75	0.016			1.5	0.48								1.95		
4	0.001	0.29	32.0	0.9			1.0	1.0	0.37	0.016			1.5	0.48								1.95		
5	0.001	0.29	32.0	0.9	+		1.0	1.0	0.37	0.048			1.5	0.48								1.95		
6	0.001	0.29	32.0	0.9	+		0.6	2.0		0.032			1.5	0.48								1.95		
7	0.001	0.29	32.0	0.9	+	+	0.3	2.0		0.016			1.5	0.48								1.95		
8	0.001	0.29	32.0				1.0	2.0		0.048			1.5	0.48								1.95		
9	0.001	0.29	32.0	0.9			1.0	2.0					1.5	0.48								1.95		
10	0.001	0.29	32.0	0.9	+		2.0	2.0					2.0									1.95		
11	0.001	0.29	32.0	0.9	+	+	2.0	2.6		0.010			2.0		0.080	0.02						1.625		
12	0.001	0.29	32.0	0.9	+	+	2.0	2.6					2.0		0.065	0.02	1.2	+				1.625		
13	0.001	0.29	32.0	0.9	+	+	0.6	2.6					2.0		0.048	0.01	4.0	+				2.275		
14								2.6					0.3		0.016		4.0	+				0.975		
15								2.6							0.065		2.6	+						
16				0.3		−		2.6										+						
17				0.9				2.6										+						
18				0.9				2.6							0.065			+						
19				0.9				2.6										+						
20				0.9		−		2.6										+						
21				3.0				2.6										+						
22				2.4				2.6										+						
23					+	+		2.0				+						+	−					+
24																								
25																								

CHEMICAL DISCUSSION

In these patients, spectroscopic examination of the blood showed that the abnormal appearance of the specimens was associated with an absorption band in the red part of the spectrum. When the blood was diluted just enough to allow for the passage of light, this band divided the red-orange field into two parts occurring roughly in the ratio of 55:45, the line being slightly nearer the yellow end. On spectrometric measurement the density center of the absorption band, which was not sharply defined, was located at 634 millimicrons. This is the position given for methemoglobin by Bodansky.[1] Since Koch[2] places this band at 631 and Haurowitz[3] at 625 millimicrons, the spectrometer was recalibrated by means of emission spectra and the position of the absorption bands of oxy-, carboxy-, met-, and sulf-hemoglobin verified.

Since at the time of our observations (January, 1937) the only abnormal pigment associated with sulfanilamide therapy was sulfhemoglobin, particular care was taken to ascertain the nature of the pigment encountered in our patients. That it was not sulfhemoglobin was demonstrated in several ways. Not only was the latter available for comparative study in a patient who had taken large and oft-repeated doses of acetanilide, but it was also prepared in the laboratory. Among the various bright lines used for calibrating the spectrometer was the broad band in the red given by calcium. This extends from 618 to 626 millimicrons which, in our hands, is precisely the position of the absorption spectrum of sulfhemoglobin. In recent investigations, Paton and Eaton[4] report 615 to 628 millimicrons. According to Kahn and Goodridge,[5] the characteristic range is from 610 to 625 millimicrons; Jean Prevot[6] located it at 610-620, Haurowitz[3] at 617 millimicrons. When this pigment was observed in a hand spectroscope under such conditions of dilution that only red and orange could be seen, the line divided the field into distinctly uneven parts, roughly in the ratio of 70:30, the narrower including the orange. Addition of ammonia or dilute ammonium sulfide did not affect the band produced by sulfhemoglobin, whereas it caused the disappearance of the band associated with sulfanilamide therapy. Likewise, 1 per cent solution of potassium cyanide caused the immediate disappearance of the line ascribed to methemoglobin, whereas it only slightly affected the sulfur derivative. Passage of illuminating gas through solutions containing sulfhemoglobin shifted the absorption band from 615 to 631 (maximum 622) to 611 to 624 (maximum 617) millimicrons. Clarke and Hurtley[7] regarded this shift as evidence of a new compound, carboxysulfhemoglobin, and recorded the change in position of the absorption band as from 610-625 to 605-620 millimicrons. The line obtained with the pigment responsible for the cyanosis following sulfanilamide therapy disappeared on treatment with illuminating gas.

The response to illuminating gas is disconcerting since stability to carbon monoxide is regarded as one of the primary characteristics of the pigment. Nevertheless, dilution with water also caused the absorption band to disappear. Furthermore, the methemoglobin did not persist in the unhemolyzed sample, but slowly reverted to normal blood pigment. This was evidenced not

only by a change in the spectroscopic findings but also by disappearance of the typical "dirty" color of the blood sample. Because of this fading of the characteristic band, the spectroscopic examination of the blood was carried out immediately. In three of our cases the absorption band vanished completely within periods varying from five to fifty minutes once a suitable concentration for observation had been achieved. Excessive dilution was accompanied by fading within a few seconds. Addition of a few drops of the sample restored the band only to have it disappear again, though more slowly. In one instance, the absorption band persisted overnight. This behavior was noted by a varied group of curious observers who, without instruction or assistance, were asked to describe what they saw in the spectroscope.

The instability of methemoglobin is by no means a new idea. According to Stadie[8] "methemoglobin disappears from the circulation with great rapidity whether it is introduced by injection of methemoglobin or formed within the circulation by the action of chemicals or of pneumococci." Harrop and Waterfield[9] noted the "great instability of this pigment in the blood stream" and stated that "unless it is produced very rapidly it is destroyed before it is formed in appreciable amounts."

It should be emphasized that we have examined spectroscopically many specimens of blood from patients on sulfanilamide therapy who were slightly cyanotic without detecting abnormal pigment. Frequently, less than thirty seconds elapsed from withdrawal of the blood to its dilution and examination. A few samples were tested without dilution by allowing a drop of blood to fill a thin wedge-shaped chamber formed by holding two microscope slides together with a wire clip, a fragment of broken slide separating them at one end; the hand spectroscope was applied to that part of the wedge which blotted out all but the red-orange part of the spectrum.

Although it is probable that as little as 5 per cent saturation of the blood with methemoglobin will suffice for visual detection of darkening of the specimen, much larger concentrations are required for observation and measurement of its absorption band. According to Kobert (1902, cited by Peters and Van Slyke[10]) ordinary spectroscopic examination is uncertain when less than 25 per cent of the blood pigment is in the form of methemoglobin. Bloem,[11] in carefully controlled experiments, ascertained that the lower limit of detectability for methemoglobin was 0.3 gm. per 100 c.c. of blood when diluted 1:5 and observed through tubes of 1 inch bore against a 60-watt frosted bulb. Bensley and Ross[12] found 0.4 to 0.9 gm. per 100 c.c. to give a very faint band; over 2 gm. of methemoglobin were required to obtain an absorption band designated as "one plus."

Although earlier investigators consistently reported sulfhemoglobin, more recent papers demonstrate the presence of methemoglobin. The literature repeatedly warns against the concurrent use of sulfates and sulfanilamide. Present evidence, however, is wholly inadequate to demonstrate that sulfhemoglobin is formed from sulfates or, for that matter, from sulfanilamide. Both compounds contain oxidized sulfur, whereas sulfhemoglobin is known

to be produced only in the presence of reduced sulfur. In the hemoglobin derivative the sulfur is negative; in these therapeutic agents sulfur functions as a positive element. As far as is known, sulfates are absorbed and excreted unchanged by the kidneys. Sulfanilamide as such has been shown to occur in urine.

Sulfhemoglobin is not even produced, ordinarily, by inhaling hydrogen sulfide because of the rapid oxidation to sulfate in the blood stream. Yet sulfhemoglobin is promptly formed when only traces of hydrogen sulfide are available once the normal blood pigment has been altered (presumably to methemoglobin) by certain drugs which are chemically related to aniline. A single large dose of acetanilide, for instance, may suffice to produce methemoglobin. Its continued use, however, leads to the formation of sulfhemoglobin, the necessary reduced sulfur being secured from the intestinal tract where hydrogen sulfide may have accumulated due to stasis or to an overabundance of sulfur-containing foods. A patient, therefore, who needs catharsis and fails to get it because of an interdiction against sulfates will still develop sulfhemoglobinemia provided the sulfanilamide "sensitizes" his blood pigment and his diet contains sulfur capable of yielding hydrogen sulfide.

It should be recognized that cyanosis associated with methemoglobinemia is temporary and responds promptly to withdrawal of the causative agent. Except in very mild cases, cyanosis occasioned by sulfhemoglobin persists for weeks since the pigment is extraordinarily stable both in the blood stream and in the laboratory. Although the cyanosis associated with sulfanilamide therapy may cause no clinical concern so long as it is due to methemoglobin, the possibility of the development of sulfhemoglobinemia should not be ignored. Sulfhemoglobin (never methemoglobin in our experience) may be detected by spectroscopic examination of a transilluminated ear lobe.

Because of the slight degree of cyanosis occurring in anemic patients with met- and sulf-hemoglobinemia, we wish to stress the warning sounded by Discombe.[13] It is unusual for a patient with less than 5 gm. of hemoglobin per 100 c.c. of blood to become cyanotic.[14] This is regarded as the "threshold value" for the appearance of cyanosis. Since it is the absolute level of non-oxygenated hemoglobin which determines the presence or absence of cyanosis, sulfanilamide administration must be undertaken cautiously when the total hemoglobin concentration is low. If the erythrocyte count is normal and *remains so* (twice weekly checking is advisable), the appearance of definite cyanosis is a satisfactory signal for temporary cessation of the therapy. With anemic patients, however, this warning is lacking. Spectroscopic examination may not suffice to indicate danger.

SUMMARY

1. Cyanosis may exist as a result of sulfanilamide therapy without the presence of abnormal pigment being revealed by spectroscopic examination.

2. The methemoglobin encountered in the cases described was highly unstable, thus interfering with detection and quantitative determination.

3. The abnormal pigment, although very unstable in vivo, persisted during oxygen administration but promptly disappeared upon discontinuance of the drug.

4. Some of the properties of met- and sulf-hemoglobin are described.

CONCLUSION

The occurrence of cyanosis due to methemoglobin in four cases is presented as a specific effect of sulfanilamide therapy.

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HYPERINDICANEMIA IN RENAL INSUFFICIENCY AND THE SIGNIFICANCE OF THE DIAZO REACTION*

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IN 1911 Obermeyer and Popper[1] demonstrated that indican was increased in the blood of uremic patients. Since then hyperindicanemia in renal insufficiency has been observed and confirmed by many investigators.[2-13] From a review of this literature it is generally assumed that the normal range of plasma indican content is from 0.026 mg. per cent to 0.086 mg. per cent, although Haas,[12] in studying cases of intestinal putrefaction, has shown that

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the plasma indican may rise as high as 0.15 mg. per cent and Rosenberg[13] has demonstrated an increase as high as 7.0 mg. per cent in the plasma from cases in renal insufficiency.

In addition to the diagnostic and prognostic significance of hyperindicanemia, it has been suspected that indican might play a rôle in the production of uremia. This was emphasized when Harrison,[14] Hewitt,[15] Bolliger and Earlam[7, 9] and others,[16-19] pointed out that the diazo reaction of Andrewes[17] occurred only in uremia, and Bolliger and Earlam[7] claimed that the diazo reaction might be due to or dependent upon the indican content of plasma.

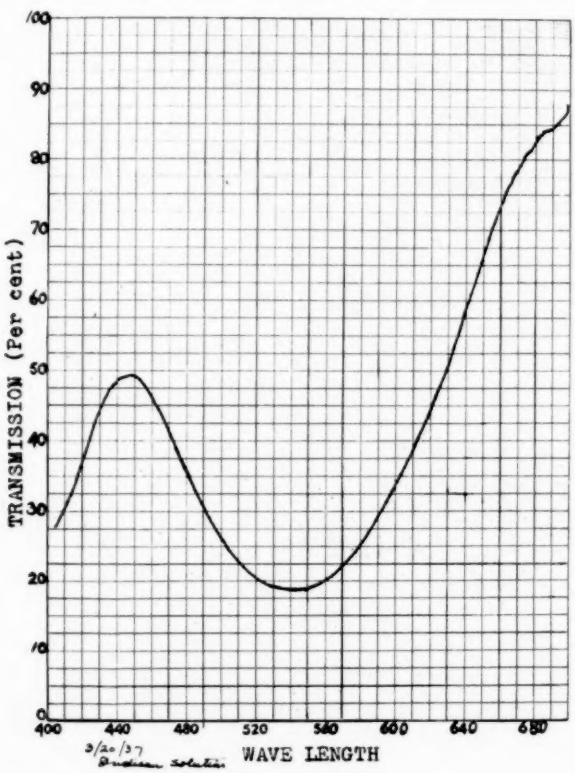

Fig. 1.—Spectrophotometric absorption curves of chromogen developed from indican in solution. A comparison of the curves obtained using blood plasma and urine show that all three are identical. These curves were obtained with the recording spectrophotometer of the Color Measurement Laboratory of the Massachusetts Institute of Technology.

The prognostic significance attached to the diazo reaction, therefore, might well be the same as that of hyperindicanemia. No great consistency, however, has been demonstrated in the results of past studies on indican, and the diazo reaction has been thought by some to be due, not to indican, but to such substances as the cyclic amines and the aromatic oxyacids.

In the light of such uncertainty it appeared that this subject deserved further consideration, and we, therefore, undertook to investigate the various methods of indican assay and the determination of the method most reliable.

Early in our work it was evident that Sharlit's method[20] was specific for the determination of indican in the blood, as well as urine,[21] and was more sensitive than the procedures more commonly used. Further evidence for this lies in the identity of spectrophotometric absorption curves of indican in solution (see Fig. 1) and of the chromogen developed from blood plasma and urine.

Sharlit's results, while closer to the actual values than others, are somewhat inaccurate because of the use of cobalt sulfate ($CoSO_4.7H_2O$) as a standard for color comparison. Furthermore, the correction factor used in the original method could hardly give the desired accuracy. Nonspecific variables, such as glucose, play little or no part in the development of the color, except where low values are to be expected.

All workers in this field are aware of the fact that pure indican itself is not readily available and that errors may be derived from the use of standards other than indican. Moreover, if the plasma indican values in renal insufficiency are to be of any significance, one should have a method sensitive enough to measure the indican content in normal individuals where the amounts have been shown to be small. We have been able to demonstrate that indican is a normal constituent of blood, present even in children as young as three months of age.

METHODS

Using the Evelyn photoelectric colorimeter,[22] Sharlit's method was employed, using an appropriate filter transmitting the maximum amount of light at the wave length of 5,400 $\mu\mu$. The choice of the filter for such a system was determined by the fact that the color developed has its maximum optical density at 5,400 $\mu\mu$, as shown by the recording spectrophotometric absorption curves.

For purposes of convenience the method employed is presented in brief. The few changes that have been made from the procedure as described originally[22] were necessitated for color measurement by the Evelyn photoelectric colorimeter.

Two cubic centimeters of plasma were diluted with 3 c.c. of distilled water and deproteinized by precipitation with 5 c.c. of 25 per cent trichloracetic acid. To 5 c.c. of the clear filtrate (the aliquot being equivalent to 1 c.c. of plasma) in a calibrated centrifuge tube were added 5 drops of a 1 per cent solution of potassium persulfate and 0.5 c.c. of 1 per cent thymol in 95 per cent ethyl alcohol. This was shaken, and 5 c.c. of 12.5 per cent trichloracetic acid in concentrated HCl were added. The solution was then corked, inverted, and allowed to stand for fifteen minutes at room temperature, and then placed in a boiling water-bath for twelve minutes. After standing again at room temperature for twenty-five minutes, it was centrifuged for ten minutes. The supernatant fluid was carefully pipetted off, and the volume made up to 12 c.c. with glacial acetic acid. On stirring a perfectly transparent color developed.

TABLE I

CASES	INDICAN MG. % AVERAGE	INDICAN MG. % RANGE	B.U.N. MG. % AVERAGE	B.U.N. MG. % RANGE	NONPROTEIN NITROGEN MG. % AVERAGE	NONPROTEIN NITROGEN MG. % RANGE	INTRAVENOUS P.S.P. % AVERAGE	INTRAVENOUS P.S.P. % RANGE	DIAZO REACTION
25 normals	0.26	0.08-0.50							
14 cases with various diseases without nephritis	0.38	0.21-0.90	16	11-28	33	29-37	-	- -	0
13 hypertensives without nephritis	0.32	0.14-0.48	12	10-19	-	- - -	62	50-85	0
Nephrotic syndrome	0.22		29		41		50		0
Nephrotic syndrome	0.86		25		39		20		0
	1.14		32		-		15		0
Nephrosis	0.20		12		23		75		0
Subacute nephritis	1.24		44		65		0		0
Subacute nephritis	3.80		80		93		0		
	4.20		123		150		0		

Twenty-Six Cases of Chronic Glomerulonephritis

CASE NO.	INDICAN MG. %	B.U.N. MG. %	N.P.N. MG. %	INTRAVENOUS P.S.P. %	DIAZO REACTION	REMARKS
6	0.14	--	28	--	Negative	
7	0.18	--	31	--	Negative	
8	0.18	11	--	75	Negative	
	0.40	13	--	70	Negative	
9	0.24	13	26	55	Negative	
10	0.24	54	--	20	Negative	
	2.50	155	--	0	Positive	Died
11	0.26	11	--	45	Negative	
12	0.28	15	--	65	Negative	
13	0.30	24	--	40	Negative	
	0.48	18	--	50	Negative	
14	0.30	18	--	--	Negative	
	0.56	22	31	60	Negative	
15	0.38	16	--	55	Negative	
16	0.40	8	--	50	Negative	
17	0.40	12	--	60	Negative	
18	0.40	17	--	55	Negative	
19	0.40	44	--	--	Negative	
	0.48	43	--	--	Negative	
20	0.58	13	--	40	Negative	
21	0.66	30	--	20	Negative	
	1.36	51	--	15	Negative	
22	0.92	27	46	50	Negative	
23	0.80	66	77	spt.	Negative	
	1.20	77	--	0	Negative	
24	1.60	83	--	spt.	Positive	
	3.20	115	--	0	Positive	Died
25	1.68	74	--	0	Positive	
	4.20	71	--	0	Positive	
26	2.80	123	--	spt.	Positive	Died
27	2.90	114	--	spt.	Positive	
	3.36	153	--	0	Positive	
	1.90	128	--	5	Positive	
	2.16	117	--	spt.	Positive	
28	3.40	113	--	0	Positive	
	4.30	126	--	0	Positive	
29	3.88	119	130	spt.	Positive	
	3.78	104	--	0	Positive	
30	4.40	200	--	0	Positive	Died
31	4.48	124	--	0	Positive	Died

TABLE I—CONT'D

Nine Cases of Chronic Vascular Nephritis

CASE NO.	INDICAN MG. %	B.U.N. MG. %	N.P.N. MG. %	INTRAVENOUS P.S.P. %	DIAZO REACTION	REMARKS
32	0.24	13	--	35	Negative	
33	0.60	44	60	3	Negative	
	1.16	48	66	0	Negative	
34	0.96	16	--	-	Negative	
35	1.00	33	--	20	Negative	
	1.04	25	--	-	Negative	
	0.82	42	--	-	Negative	
36	1.24	123	--	-	Negative	
	1.80	136	141	0	Positive	Died
37	1.40	33	--	10	Negative	Died
38	1.60	91	119	0	Positive	Died
39	1.40	81	--	2	Negative	
	2.60	95	--	0	Positive	
	2.38	105	--	0	Positive	
40	1.80	61	--	0	Positive	Died
			Eight Cases With Chronic Vascular Nephritis and Cardiac Decompensation			
41	0.20	15	--	--	Negative	Heart compensated
42	0.36	61	--	5	Negative	Decompensated
43	0.40	11	--	65	Negative	
44	0.62	25	--	15	Negative	Decompensation slight
45	0.74	--	94	--	Negative	Cerebral hemorrhage
	1.24	127	151	--	Negative	Died
46	0.74	25	--	28	Negative	Uncontrolled diabetes
47	1.06	82	110	0	Negative	Decompensated
	2.16	126	133	0	Positive	Died
48	1.80	72	--	0	Positive	
	1.20	50	--	5	Negative	
			Six Cases With Other Renal Pathology			
49	0.48	37	50	15	Negative	
	0.56	26	-	-	Negative	Hydronephrosis
50	0.34	18	-	-	Negative	Cancer of the prostate with acute retention
51	1.00	-	72	-	Negative	Enlarged prostate with
	0.60	-	41	-	Negative	acute retention relieved
	0.36	-	34	-	Negative	by operation
52	0.74	-	46	-	Negative	Left nephrectomy and
	0.80	-	54	-	Negative	right pyelonephritis
53	1.50	59	76	-	Positive	Pyelonephrosis in remain-
	1.60	64	-	0	Positive	ing kidney and cardiac decompensation
54	0.56	71	82	5	Negative	Acute cholecystitis and
	0.90	36	60	10	Negative	question of hepato-
	0.34	15	36	32	Negative	renal syndrome

The solution was transferred to standard Evelyn colorimeter tube and placed in the colorimeter using a filter (Corning glass) transmitting between 5,200 $\mu\mu$ and 5,700 $\mu\mu$, with its maximum transmission at 5,400 $\mu\mu$. Readings were made from the galvanometer (1 mm. to 0.027 microamperes) when the maximum deviation of the string had occurred. The result was read directly in milligrams per cent from a calibration curve previously obtained by plotting the galvanometer deflections from a series of known amounts of indican*

*Indican was obtained from E. Merck, Darmstadt, Germany, and from Dr. Fraenkel and Dr. Landau, Berlin, Germany.

used as standards, the amounts varying between 0.1 mg. per cent and 6.0 mg. per cent. The calibration curve obtained is shown in Fig. 2, the galvanometer readings (G) being plotted against milligrams indican per 100 c.c. of blood. This curve when plotted on semilogarithmic paper is a straight line, hence the value of X (milligrams indican) can be calculated by the following formula:

$$X = \frac{(2 - \log. G)}{K_2} \times 100 \text{ where } K_2 = 15.2,$$

provided the various volumes stated in the method are adhered to.

The patients studied comprised a large group from the Renal Vascular Clinic of the Peter Bent Brigham Hospital. All the patients had been under observation for long periods of time and their status was well known. The patients who were in a more advanced stage of their disease or in a critical condition, were for the most part from the wards of the hospital and in some cases the diagnosis was confirmed by postmortem examination.

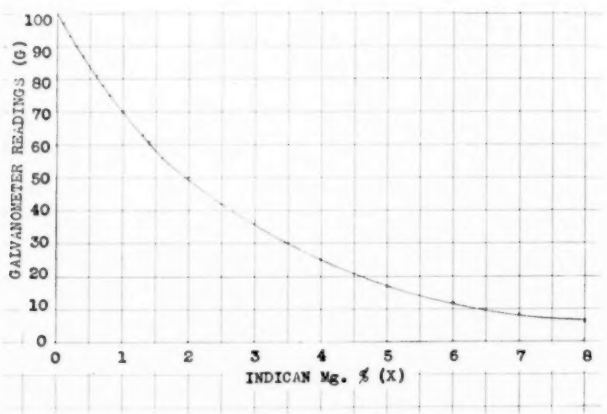

Fig. 2.—Calibration curve for the determination of indican in blood plasma in milligrams per cent (aliquot of indican solution equivalent to 2 c.c. blood plasma).

RESULTS

One hundred and six patients were studied with 164 determinations of the plasma indican content. The majority of the patients excluding 25 normals, used as controls, had parallel determinations of blood urea nitrogen or nonprotein nitrogen, phenolsulphonephthalein excretion, and diazo reaction in addition to the assay of the plasma indican, for purposes of correlation. The results are condensed in Table I.

The 25 normals showed an average plasma indican of 0.26 mg. per cent, with a range from 0.08 mg. per cent to 0.50 mg. per cent, which is considerably higher than values that have been usually quoted, confirming Sharlit's contention that the plasma indican content is higher than commonly assumed. In 14 cases with miscellaneous disease including syphilis, pernicious anemia, cirrhosis of the liver, bronchopneumonia, gastric ulcer, nonspecific enteritis, uncontrolled diabetes, cancer of the liver, and one case of subarachnoid hem-

orrhage, the average was 0.38 mg. per cent, with a range from 0.21 mg. per cent to 0.90 mg. per cent. The highest indicans in this group were in a patient with untreated pernicious anemia and in a moribund man with carcinoma of the liver. In these cases the plasma indican was 0.60 mg. per cent and 0.90 mg. per cent, respectively. All of these patients had normal blood urea nitrogen values, except one with subarachnoid hemorrhage whose B.U.N. was 28 mg. per cent; curiously his indican was within normal limits. None of these patients had a positive diazo reaction.

Another group of 13 cases of vascular hypertension showed a plasma indican average of 0.32 mg. per cent, an average blood urea nitrogen of 12 mg. per cent, a P.S.P. excretion of 62 per cent, and negative diazo reactions.

The most interesting group consisted of 43 cases of nephritis of various types as shown in Table I. In this group it will be noted that the plasma indican is elevated above normal limits or above the values found in the various unrelated diseases when there is also some elevation of the blood urea

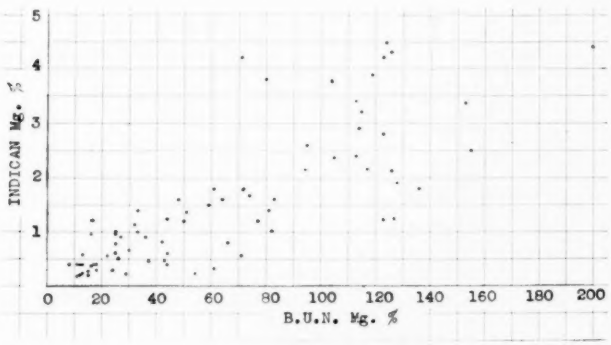

Fig. 3.—Figure showing the relationship of B.U.N. milligrams per cent to indican milligrams per cent.

nitrogen and some impairment of the phenolsulphonephthalein excretion. The lowest value of the plasma indican was 0.14 mg. per cent and 4 cases with values above 4.0 mg. per cent, the highest determination being 4.48 mg. per cent.

In practically all the cases with an increased blood urea nitrogen the plasma indican was elevated, although there is no direct relationship as shown in Fig. 3. Similarly, the increase in the plasma indican was usually associated with an impaired phenolsulphonephthalein excretion, but here the relationship was even less definite, due mainly to the inherent errors associated with phenolsulphonephthalein studies.

Another point of importance is that all patients with a plasma indican of over 1.50 mg. per cent exhibited a positive diazo reaction and that 10 of 16 patients with such a positive reaction died, while the other six are all in a critical state with a poor prognostic outlook.

In regard to symptomatology, we could find no definite correlation between the onset of nausea, vomiting, muscular twitching, convulsions or coma, and the rise in the blood indican.

In a study of 6 patients with kidney involvement other than nephritis, the plasma indican was elevated when there were other signs of retention of metabolic products, but once the condition was relieved by surgery or other treatment, the indican level decreased to normal along with the return of adequate renal function.

Similarly, in another group of cases with chronic vascular nephritis complicated with cardiac decompensation it was found that when renal insufficiency existed the blood indican was elevated to moderately high levels. With restoration of cardiac compensation, the blood indican decreased to a level consistent with the degree of renal insufficiency. In one of these cases the diazo reaction became negative.

The fact that the diazo reaction, as performed by Hewitt,[15] was positive in those cases with an indican content above 1.50 mg. per cent was interesting and suggestive. It has been said that the diazo reaction is due to or dependent on the indican content of blood. To check this we made known solutions of indican in distilled water and in blood plasma in concentrations of 0.2 mg. per cent to 3.0 mg. per cent. The diazo reaction in these dilutions became positive at 1.5 mg. per cent. The pink color in this dilution was just perceptible and did not persist, but in a concentration of 1.8 mg. per cent the color was less fleeting and at 2.0 mg. per cent the color persisted for a definite interval.

We then submitted solutions of indican, indican in blood plasma, alcoholic extracts of urine and blood plasma in which the indican content was known to be high, also to the diazo reactions and secured spectrophotometric curves which definitely demonstrated that the colors developed were similar. It is difficult to obtain a satisfactory absorption curve of the diazo reaction in a sample where the reaction is not pronounced and also fades rapidly, but in those with an extremely high indican and consequently a more persistent diazo reaction, the absorption curves leave little doubt as to the similarity of the curves obtained to those in which known indican was added. From this we conclude that the development of color in the diazo reaction is dependent upon an increased indican content of the blood plasma of patients.

DISCUSSION

In the past numerous investigators have suggested that the determination of indican in blood was an excellent test of renal insufficiency and, in many cases, a more reliable index than blood urea nitrogen or nonprotein nitrogen. Interpretation of our data does not allow such a sweeping statement. Indican is increased in the blood of patients with renal insufficiency, but it is associated usually with an elevation of the blood urea nitrogen and a decreased phenolsulphonephthalein excretion. In a certain group of cases where extrarenal factors have given rise to renal impairment, the indican is temporarily increased, but with improvement in the patient, the indican returns to a lower level as do the other retained metabolic products, such as urea nitrogen. From the data obtained it appears that an elevation of indican above a level of 0.50 mg. per cent may be taken as an indication of renal

insufficiency. However, the determination of indican adds little if anything to the study of these patients from a diagnostic point of view.

The diazo reaction has already been set forth as a prognostic test in nephritis by other workers, and when positive, it is generally accepted that the prognosis is poor and in most cases fatal. In our limited experience this fact has been confirmed since 10 of 16 patients with a positive diazo reaction, at the time of writing, are dead, and the others are all in a critical condition with a poor prognostic outlook. The majority of our cases had the positive reaction only over a relatively short period of time, but rare cases of chronic glomerulonephritis have been reported where the positive diazo reaction has been noted over a long period of months.[18, 19]

In our experiments, spectrophotometric analysis demonstrated that the chromogen of the diazo reaction on plasma of patients with a high plasma indican was identical with the curves obtained on pure solutions of indican made up to those concentrations which exist in the blood of uremic patients. This seems to be conclusive proof that the diazo reaction is dependent on a high plasma indican. Further, it has been demonstrated that this level must be 1.5 mg. per cent or higher before a positive reaction is noted.

If these conclusions are correct, the diazo reaction, by reason of its simplicity, becomes the practical clinical test of choice for it both indicates that the plasma indican is elevated to at least three times the normal range and that a fatal termination may be expected in a relatively short period of time.

It has not been possible to demonstrate a definite correlation between an increase of the plasma indican and the symptomatology of uremia, and it is felt that indican is probably not a toxic substance. This statement seems justified since Earlam and Bolliger[9] have shown that the presence of indican in the plasma of dogs suffering from renal insufficiency, in amounts several times greater than the highest yet recorded in fatal (uremic) cases, caused no untoward effects whatever.

CONCLUSIONS

A reliable method for indican determinations has been adapted to a photoelectric colorimeter, thus obviating the constant use of indican as a standard and removing the inherent errors of ordinary color comparison colorimetry.

Indican, a normal constituent of blood, is elevated in cases with renal insufficiency which also exhibit a retention of nitrogenous products and an impaired phenolsulphonephthalein excretion.

The diazo reaction is due to the presence of indican and becomes positive when the level of the latter reaches 1.5 mg. per cent and is a more useful practical test than indican assay in renal insufficiency because of the ease of determination and the prognostic import that it conveys.

There appears to be no definite correlation between the symptomatology of uremia and the raised plasma indican.

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TRANSIENT ELECTROCARDIOGRAPHIC CHANGES NOTED DURING ATTACKS OF ANGINA PECTORIS WITH REPORT OF A CASE*

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THE opportunity of obtaining an electrocardiogram during the acute phase of an anginal seizure does not often present itself. For obvious reasons, one hesitates to induce an attack of angina pectoris simply for the purpose of securing such a record and consequently the literature contains relatively few reports dealing with such phenomena, particularly cases in which syphilitic aortitis and coronary thrombosis can be definitely ruled out. The changes in the electrocardiogram, although transient, are so striking and in general so consistent as to be of considerable clinical interest. The cases which have been reported to the present time deal almost entirely with alterations in the standard limb leads, and so far as we have been able to find none include a study of the chest lead. In the case presented here all four leads were obtained before, during, and after the anginal seizure, thus permitting a study of the changes occurring in the chest lead as well as in the three standard leads.

The changes which have been commonly reported[1-6] in uncomplicated angina pectoris tracings consist of deviation of the RS-T segment and alteration in the amplitude and direction of the T-waves. In the observations of Feil and Siegel[2] the most outstanding finding was a definite depression of the RS-T segment in Leads I and III with a prompt return to its previous status after cessation of the pain. Parkinson and Bedford,[1] in the study of five cases, noted depression of the RS-T segment and decreased amplitude or inversion of the T-waves in one or two leads followed by almost complete disappearance of these changes after the seizure had passed off. Brow and Holman[4] find that the most important electrocardiographic changes consisted of "alteration in the terminal deflections."

CASE HISTORY

C. M., a white male, aged 43 years, was admitted to the Wisconsin General Hospital on November 2, 1936, complaining of "pain beneath the breast bone." He dated the onset of this condition to five years ago when he was suddenly seized with an attack of substernal pain, epigastric discomfort, and nausea coming on about one-half hour after shoveling snow. Belching of gas a short while later relieved his symptoms entirely. Similar episodes occurred intermittently until three months previous to admission, since which time the symptoms had become more severe and more frequent, coming on after the slightest exertion, usually accompanied by vomiting, dysphagia, palpitation, and dyspnea. Laryngoscopy, chest, gall bladder, and stomach x-ray studies repeated at intervals had been negative. The past medical history

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revealed the occurrence of measles, tonsillitis, mumps, smallpox, influenza, whooping cough, pneumonia, and pleurisy in early life. The family history was interesting in that his mother died at the age of 58, with a condition similar to that of the patient.

To physical examination the patient showed slight tortuosity of the retinal vessels and moderate peripheral sclerosis. The pulse was regular at a rate of 72. The blood pressure was 122/70. There was no cardiac enlargement on percussion. The heart sounds were of fairly good quality at all areas and no murmurs were heard. The lungs and abdomen were negative, and there was no edema of the extremities.

The results of the routine laboratory studies were within normal limits. X-ray examination of the esophagus and gastrointestinal tract revealed no abnormalities. Orthodiascopic studies showed the heart to be within normal limits as to size and shape.

On November 9 the patient was sent to the cardiology department for an electrocardiogram. The exertion incident to the trip to the laboratory precipitated an anginal attack during which the electrocardiogram, a portion of which is reproduced in Fig. 1, was recorded.

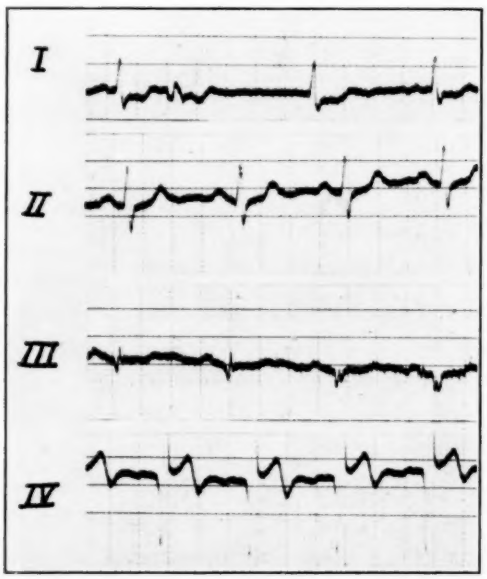

Fig. 1.—Electrocardiogram taken on Nov. 9, 1936, during the initial anginal attack precipitated by walking to the electrocardiographic laboratory. Note, especially, the diphasic T-4.

The complete record showed two ventricular extrasystoles in Lead I, followed by sinoauricular block with auriculoventricular nodal escape. The dominant rhythm was a sinus arrhythmia at a rate of 75 to 90. There was a large Q_3, depression of the RS-T segments in Leads I and II, elevation of the RS-T segment in Lead IV, and a diphasic T_1 and T_4.

From the standpoint of scientific interest it was felt desirable to obtain a series of tracings before, during, and after such an attack, in an attempt to determine whether these changes could be consistently reproduced. With the permission and cooperation of the patient such a series was obtained two days later. The initial control electrocardiogram taken with the patient symptomless is shown in Fig. 2*A*. It will be noted that this tracing exhibited a deep Q_3, slight depression of the RS-T segment in Lead I, and slight elevation of the RS-T segment in Lead III. T_1 and T_2 were almost flat, and T_4 was normally inverted. These changes will be recognized as characteristic of coronary sclerosis. The patient was then asked to walk up a short flight of stairs, an exercise which he had found almost invariably precipitated attacks of substernal pain. During the seizure which followed, the tracing shown in Fig. 2*B* was recorded. On this occasion there was a marked sinus arrhythmia with a rate

varying from 45 to 90. The RS-T segment became more definitely depressed in Lead I and elevated in Lead IV. T_1 and T_3 became diphasic and somewhat increased in amplitude, T_2 deeply inverted, and T_4 diphasic but chiefly upright. Five minutes later the symptoms had completely disappeared and the electrocardiogram taken at this time, shown in Fig. 2*C*, revealed almost complete return to the preanginal status, with the exception of very slight inversion of T_1 and a slight increase in the amplitude of T_2 and T_3.

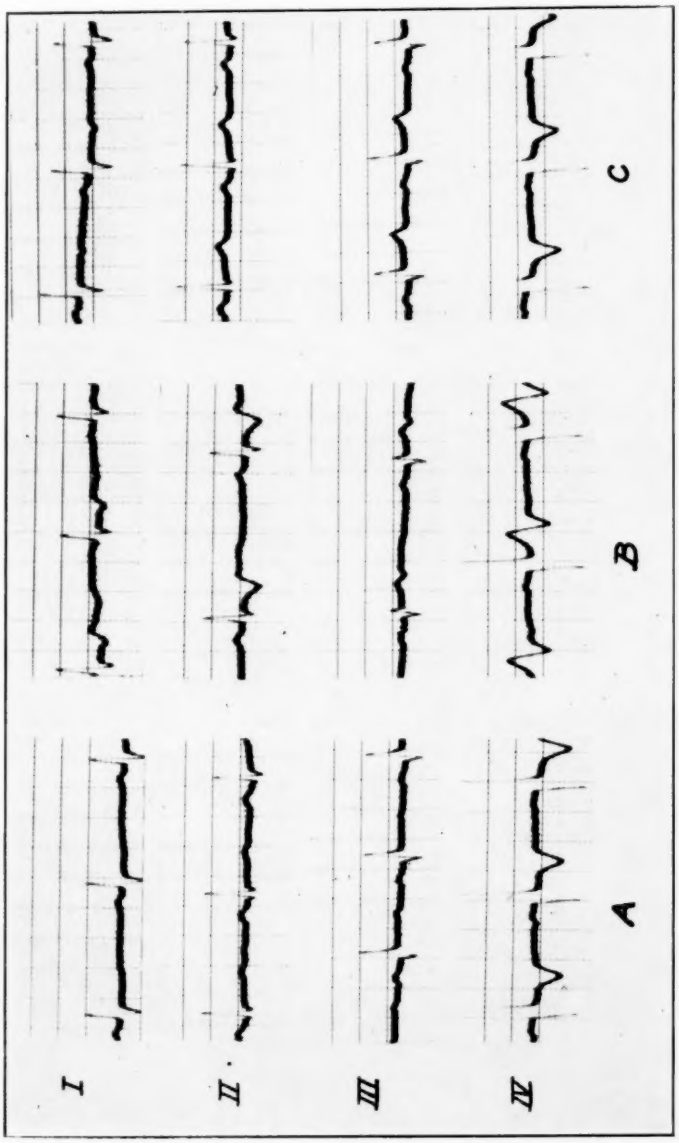

Fig. 2.—Electrocardiogram taken on Nov. 11, 1936. *A*, Before anginal attack. Note deep Q-3. Practically isoelectric RS-T segments. Almost flat T-1 and T-3, T-4 normally inverted. *B*, Taken a few minutes later during anginal attack. RS-T-1 more definitely depressed. RS-T-3 more definitely elevated. T-1, T-3, and T-4 diphasic, T-2 diphasic, but chiefly inverted. *C*, Taken five minutes later after symptoms had entirely disappeared. Tracing has returned very nearly to preanginal status. T-4 has resumed its inverted position.

DISCUSSION

The changes seen in the electrocardiogram during a seizure of typical angina pectoris are recognized as being practically identical with those encountered following acute coronary occlusion, differing only in the point of

duration. In the case presented this was further borne out by the changes in the chest lead characterized by the deviation of the RS-T segment and a tendency for the T-wave to turn upright. This lends further support to the now generally accepted view that the symptoms of coronary insufficiency are due primarily to myocardial ischemia of a transient nature in angina pectoris and of considerable duration following coronary occlusion.

SUMMARY

A case is presented in which electrocardiograms were taken before, during, and immediately following an attack of typical angina pectoris. The chest lead was taken in addition to the three standard limb leads, and the changes in the tracing taken during the attack were characteristic of acute coronary occlusion with the exception of their transitory nature.

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THE PARTITION OF NITROGEN IN CANINE GASTRIC JUICE

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THE partition of nitrogen in the gastric juice has been the subject of much controversy. Rosemann (1927) and Carlson (1915, 1919) maintain that practically all the organic solids of the gastric juice are represented by proteins (Carlson) or "a protein-like body" (Rosemann). This view was shared by Dienst as recently as 1931. On the other hand, it has been asserted that the gastric juice, or rather the gastric contents, in normal persons and in various clinical cases contain considerable amounts of nonprotein nitrogen, ranging from 16 to 200 mg. per cent (Polland, Roberts, and Bloomfield, 1928; Martin, 1931; Mielke, 1932; Baltzer, 1934; Burger, Hartfall, and Witts, 1933; Diehl, 1936). The amount of nonprotein nitrogen in the gastric juice was found to be greatly increased in cases of achylia gastrica, or carcinoma, and particularly of nephritis accompanied by retention of nitrogen. Ammonia was

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early recognized to be a normal constituent of gastric juice, forming a considerable part of the total nitrogen content of the secretion (Nencki, Pavlov, and Zaleski, 1898). This finding was later confirmed by many other investigators (Salaskin, 1898, 1902; Carlson, 1915; Rosemann, 1927; Martin, etc.). It has been shown that the gastric juice contains a small quantity of urea (Martin, 1931; Komarov, 1931). Mielke regards all the nonprotein nitrogen of the gastric juice as urea, although his opinion was not substantiated by any experimental evidence. The presence of creatine bodies, uric acid, adenosin phosphoric acid, and amino nitrogen has been postulated by many investigators (Toni and Marciali, 1928; L. Martin; Baltzer; Diehl), but none of the substances belonging to this group was isolated from the gastric juice and identified chemically.

It should be mentioned that only one investigation—that of Rosemann (1927)—has been carried out on a large scale and with really pure gastric juice (obtained by sham-feeding from a dog with a gastric fistula and esophagotomy). Rosemann found that the organic matter in gastric juice consists practically entirely of a proteinlike body, the remainder being so minute in amount that it is not even worthy of mention. It should be noted that in all the studies in which large percentages of nonprotein nitrogen have been reported, the methods of routine blood analysis had been applied to the human gastric contents, the samples having been obtained from fasting subjects, sometimes without stimulation but more often after the injection of histamine, under normal or pathologic conditions. The wide divergences in the analytical results obtained even in the course of one and the same investigation (cf., for example, the work of Martin and of Mielke) are in all probability largely due to contamination with saliva, bile, or intestinal juice, all of which are known to contain large amounts of nonprotein nitrogen (for references, see Babkin, 1928). In the opinion of the writer the discrepancies might also be attributable to the inadequacy of the methods used. The proteins of the gastric juice are entirely different from the proteins of the blood (Webster and Komarov, 1932) and, therefore, it is questionable whether the methods used in blood analysis are suitable for the determination of nonprotein nitrogen in the gastric juice.

The main purpose in the present investigation was to study the efficacy of some of the common protein precipitants when applied to the proteins of the gastric juice, with the object of developing a convenient and yet reliable method for routine determinations of nonprotein nitrogen in gastric secretion. Among the methods tested, particular attention has been paid to the standard methods employed in blood analysis.

The partition of nitrogen in gastric juice was also studied. It was recently shown by the writer that pure canine gastric juice contains a number of physiologically active substances belonging to the group of nitrogenous bases (vitamin B, histamine, and guanidine-like bodies). Fractionation of these substances was effected mostly by the Kossel and Kutscher method, somewhat modified (Komarov, 1934, 1936). For this reason in our analysis

special emphasis has been placed on the nitrogenous bases of gastric juice and the distribution of nitrogen among the various fractions resulting when the method of Kossel and Kutscher is applied to the gastric juice.

METHODS

Gastric Juice.—The gastric juice employed in this study was obtained, in response to sham-feeding or subcutaneous injection of histamine dichloride (0.05 mg. per kg. body weight), from dogs with a gastric fistula and esophagotomy. Whenever there was the slightest suspicion that the secretion had been contaminated with food residues, blood or intestinal juices, the presence of the last-mentioned being indicated by the appearance of bile, the samples were discarded. Samples of clear gastric juice were collected at fifteen-minute intervals, immediately filtered and analyzed. In order that the effect of autodigestion might be observed, some samples of juice were allowed to stand for varying periods of time at room temperature or in the incubator at 38° C.

Protein-Free Filtrates From Gastric Juice.—The efficacy of the following procedures in precipitating the proteins of gastric juice was studied.

1. *Precipitation With Acetone.*—To the acid gastric juice (acidity about 150 m.eq. per liter), two volumes of acetone were added. Usually this was carried out in 15 c.c. centrifuge tubes, containing 10 c.c. of acetone, into which 5 c.c. of gastric juice was poured, the two liquids being thoroughly mixed. The tubes were corked and allowed to stand overnight. After the mixture had been centrifuged, the supernatant liquid was decanted and rapidly filtered through cotton wool. Allowance was made for the change in volume due to the volatility of acetone and the contraction which takes place when acetone is mixed with water. Sometimes it was found more convenient to filter the supernatant liquid into a 25 c.c. measuring flask, and to wash the precipitate on the centrifuge twice with 5 c.c. of 66 per cent acetone saturated with NaCl. The two modifications gave identical results.

2. *Coagulation by Heat.*—Five or 10 c.c. of gastric juice was placed in a 15 c.c. graduated centrifuge tube, loosely corked with a rubber stopper, and the tube was then immersed in a boiling water-bath. Complete coagulation usually occurred within two minutes, after which the tubes were immediately cooled under running water. The precipitate was removed either by centrifuging or by filtering. In order to ensure complete coagulation, the acidity of the gastric juice required to be about 150 m.eq. per liter.

3. *Precipitation With Trichloracetic Acid.*—Several procedures involving the use of trichloracetic acid have been studied.

(a) Five cubic centimeters of gastric juice were mixed with 5 c.c. of 15 per cent trichloracetic acid in a 15 c.c. graduated centrifuge tube. The tube, loosely corked with a rubber stopper, was immersed in a water-bath at 80° C. for five minutes, and then immediately cooled under running water. The precipitate was separated by centrifuging, and the supernatant fluid filtered. This procedure was soon found to be one of the most reliable.

The following procedures based on the use of trichloracetic acid were also studied, although they were soon found to be unreliable.

(b) Five cubic centimeters of 15 per cent trichloracetic acid were mixed with 5 c.c. of gastric juice and allowed to stand overnight at room temperature. In all cases only a very faint opalescence could be observed.

(c) The gastric juice was first neutralized with 0.2 N NaOH, the necessary amount of the latter having been determined previously by titration against methyl red. To 5 c.c. of neutralized gastric juice, 5 c.c. of 15 per cent trichloracetic acid was added, and the mixture allowed to stand overnight. By this method some protein was always precipitated, but the precipitation was never complete.

(d) The gastric juice was neutralized with NaOH, mixed with trichloracetic acid as in procedure (c), and the mixture was finally subjected to heating at 80° C. for five minutes. More protein was precipitated than by procedure (c), but the precipitation was never complete.

4. *A Modification of Somogyi's Procedure.*—Five or 10 c.c. of gastric juice was placed in a 25 c.c. measuring flask; 1 c.c. of 10 per cent $ZnSO_4.7H_2O$ and 2 drops of 0.5 per cent phenolphthalein were then added. 0.5 N NaOH was next added, until the mixture became slightly pink, after which 0.1 N HCl was added drop by drop (from 1 to 3 drops usually being required), until the supernatant fluid became colorless, while the precipitate still remained slightly pinkish. The volume was made up by the addition of distilled water. Filtration was carried out immediately or the next day.

5. *Tungstic Acid Precipitation.*—In the present study all attempts to utilize tungstic acid for quantitative precipitation of the proteins of the gastric juice were unsuccessful.

(a) *Precipitation at pH 6.*—In accordance with the principles of the original Folin-Wu method this was carried out as follows: 5 or 10 c.c. of gastric juice were neutralized with 0.2 N NaOH; 1 c.c. of 10 per cent sodium tungstate, and the amount of $\frac{2}{3}$ N H_2SO_4 necessary to adjust the reaction to pH 6.0, were added. In no case was any precipitation observed when pure, freshly secreted gastric juice was treated in this way.

(b) *Precipitation at pH 3.5.*—This was carried out exactly as suggested by Martin (1931).

(c) *Precipitation at pH < 1.0.*—It was observed that an increasing amount of nitrogen was precipitated from gastric juice by means of tungstic acid as the acidity of the medium was increased. The nitrogen content of the filtrates was lowest when the following procedure was employed. To 5 c.c. of gastric juice 1 c.c. of 10 per cent sodium tungstate and 1 c.c. of 1 N sulfuric acid were added. No precipitation immediately occurred; some precipitation took place after a few hours. The mixture was allowed to stand for several days at room temperature until no more turbidity appeared overnight in the supernatant fluid after centrifugalization. The filtrate was obtained by centrifuging at high speed and then filtering.

TABLE I

NONPROTEIN NITROGEN IN GASTRIC JUICE AS DETERMINED BY THE VARIOUS METHODS STUDIED

GASTRIC JUICE			TOTAL NITROGEN (MG. %)	NITROGEN IN THE FILTRATES (MG. %)								PROTEIN NITROGEN (MG. %)
				1	2	3A	3C	3D	4B	4C	5	
							NEUTRALIZATION T.C.A.		TUNGSTIC ACID			
SAMPLES	STIMULATION	STATE OF PRESERVATION		ACETONE	HEAT COAGULATION	T.C.A. DIRECT 80°	20°	80°	AT pH 3.5	AT pH < 1.0	SOMOGYI'S METHOD	
1	Sham-feeding	Fresh	27.20	12.04	14.56	13.44	–	–	No ppt.	6.08	9.80	15.16
2	Sham-feeding	Fresh	21.30	8.10	8.10	8.10	–	–	No ppt.	–	7.60	13.20
3a	Sham-feeding	Fresh	19.20	7.02	7.42	7.38	12.40	10.70	No ppt.	4.12	5.63	13.18
3b	(Same sample)	24 hr. at 20° C.	do.	7.42	8.12	–	–	–	No ppt.	5.49	5.74	11.78
3c	(Same sample)	24 hr. at 38° C.	do.	14.00	14.00	14.00	–	–	No ppt.	8.63	11.20	5.20
4a	Psychic	Fresh	16.50	6.58	–	–	–	–	15.40	–	6.58	9.92
4b	(Same sample)	24 hr. at 38° C.	do.	13.00	13.60	13.45	13.60	–	No ppt.	–	9.94	3.50
5	Histamine: total secretion	Fresh	8.82	5.88	6.90	6.72	–	–	–	–	4.48	2.94
6	Histamine: total secretion	Fresh	8.80	6.72	7.30	7.14	–	–	No ppt.	3.79	4.76	2.08
7	Histamine: total secretion	Fresh	12.20	9.52	9.84	9.66	–	–	No ppt.	4.57	7.85	2.68
8	Histamine: secretion for first ¼ hr.	Fresh	16.80	8.96	9.80	9.66	–	–	–	–	7.84	7.84
9	Histamine (same expt. as 8): secretion for second and third ¼ hours.	Fresh	13.16	5.88	7.56	7.28	–	–	No ppt.	3.83*	4.90	7.28
10	Secretion from pouch (after a meal) — contaminated with exudate from ulcerated skin	2 weeks at 20° to 30° C.	92.00	78.40	83.20	87.40	87.80	–	56.00	48.20	56.70	?

*Nitrogen in the filtrate after precipitation with phosphotungstic acid in the presence of 5 per cent sulfuric acid amounted to 3.43 mg. per cent.

Partition of Nitrogen in Gastric Juice.—Some data (not hitherto reported) on the partition of nitrogen in gastric juice were obtained in the course of previous studies, during which several large samples of gastric juice—amounting altogether to more than 100 liters—were fractionated by different methods (Komarov, 1933, 1934, 1935, 1936). On the basis of these findings it is believed that the addition of two volumes of acetone to freshly secreted gastric juice brings about a complete precipitation of the protein material. The distribution of nitrogen, both in the precipitates and the filtrates obtained by the use of acetone, was studied on several occasions and found to be remarkably consistent. The chemical properties and the composition of the protein precipitate have been described previously; the protein was found to be a mucoprotein having much in common with the pepsin preparations described by Pekelharing (Webster and Komarov, 1932; Komarov, 1935). The present paper affords an opportunity of describing our data concerning the partition of nitrogen in the acetone filtrates, thus making the report of the analysis of several large samples of gastric juice complete. Fractionation was carried out by the method of Kossel and Kutscher, somewhat modified. The fraction of the nitrogenous bases was isolated by precipitation with phosphotungstic acid, and the purine, histidine, arginine, and lysine fractions by the silver-baryta method. Details of the procedure used have been given in the articles in which the physiologic properties of these fractions were reported (Komarov, 1933, 1934, 1936). In addition volatile bases, urea and creatine bodies were determined in the protein-free filtrate before fractionation.

Nitrogen was determined by micromodifications of the Kjeldahl method (Pregl, 1930). Urea and ammonia plus volatile bases were determined by the urease-aeration-titration method of Van Slyke and Cullen, creatine plus creatinine by Folin's open-flask method (see Peters and Van Slyke, 1932).

EXPERIMENTAL RESULTS AND DISCUSSION

The analytical data obtained are presented in Tables I and II. Table I shows the amount of nitrogen present in the filtrates obtained by the methods outlined above. Table II shows the partition of nitrogen in the filtrates obtained by the acetone method from large samples of freshly secreted, pure gastric juice obtained in response to sham-feeding.

Of the various methods for obtaining protein-free filtrates from gastric juice that were studied in the present investigation, only the following three gave consistent and comparable results:

(1) precipitation with acetone,

(2) heat coagulation in an acid medium, and

(3) precipitation with 7.5 per cent trichloracetic acid at 80° C.

When absolutely fresh canine gastric juice was precipitated with two volumes of acetone, filtrates were obtained which, after the removal of the acetone, showed a negative biuret reaction. It was also found that the precipitates obtained from different samples of gastric juice have a consistent

elementary composition (Webster and Komarov, 1932; Komarov, 1935). On this account precipitation with acetone was selected as the basis for comparison of the efficacy of the other methods.

The average figures obtained by the various methods were computed from the data presented in Table I. Direct boiling (method 2) gives figures for

TABLE II

PARTITION OF NITROGEN IN GASTRIC JUICE OBTAINED IN RESPONSE TO SHAM-FEEDING

NITROGEN IN THE FORM OF:	SAMPLE A (4 LITERS) MG. %	SAMPLE B (6 LITERS) MG. %
Protein	18.00	19.90
Nonprotein	9.80	10.90
Urea	0.16	0.11
Creatine + Creatinine	0.09	0.11
Bases, total	5.37	6.59
Bases, volatile	1.78	2.55
Bases, nonvolatile	3.59	4.04
Bases, purine fraction	0.11	0.10
Bases, histidine-arginine fraction	1.56	1.77
Bases, lysine fraction	1.88	2.17
Monoaminoacid fraction (phosphotungstic acid filtrate)	0.70	1.02
Humine bodies	3.73	3.29

nonprotein nitrogen that are 13.5 per cent higher than those obtained by precipitation with acetone. In most cases the filtrates showed a very slight, but nevertheless distinct, positive biuret reaction, the color developed being violet blue. Precipitation with trichloracetic acid at 80° C. gives figures for nonprotein nitrogen 10 per cent higher than those given by acetone precipitation. If absolutely fresh gastric juice, obtained by means of sham-feeding, was used, the filtrates always showed a negative biuret reaction. If, however, the juice was treated with trichloracetic acid after standing for a few hours at room temperature, a slight positive (violet) biuret reaction was obtained. In "histamine" gastric juice the trichloracetic acid filtrates always showed a positive biuret reaction, the color developed being pure blue, if the juice was precipitated at once, or displaying a slightly violet tinge, if the juice was not quite fresh. In connection with these results, it should be mentioned that Pekelharing as long ago as 1902 observed some extremely interesting facts regarding heat coagulation of pepsin. He found that pepsin can be precipitated by heating only when the pepsin solution is acid and is heated to the point of coagulation very rapidly. This rule holds true for solutions of either crude or highly purified preparations of pepsin and also for gastric juice. Pekelharing also pointed out that under such conditions pepsin is partially hydrolyzed to form a product which gives a precipitate, and which he termed "*Gerinungprodukt*," and a certain albumose-like body which remains in solution. If the solution of pepsin is heated slowly, the hydrolysis is carried much further and less precipitate is formed. Under sufficiently slow heating, no precipitation occurs whatsoever. In our experiments the method of direct heat coagulation yielded filtrates showing a positive biuret reaction, and having higher contents of nitrogen than those obtained with the trichloracetic

acid procedure. These results can be satisfactorily explained by the assumption that Pekelharing's albumose is precipitated by trichloracetic acid.

Special experiments were carried out with a view to studying the influence of acidity on the completeness of heat coagulation. The results of a typical experiment of this kind are given in Table III.

TABLE III

REACTION OF GASTRIC JUICE AND PRECIPITABILITY OF THE PROTEIN MATERIAL BY HEAT

SAMPLES	GASTRIC JUICE C.C.	0.2N NaOH C.C.	CO_2-FREE H_2O C.C.	pH	NITROGEN IN THE FILTRATES MG. % OF GASTRIC JUICE	NITROGEN PRECIPITATED MG. % OF GASTRIC JUICE
1	5.00	0.00	5.00	–	16.23	13.17
2	5.00	2.00	3.00	1.80	21.00	8.40
3	5.00	2.50	2.50	2.00	22.40	7.00
4	5.00	3.00	2.00	2.70	23.50	5.90
5	5.00	3.50	1.50	3.00	25.20	4.20
6	5.00	3.60	1.40	5.40	24.40	5.00
7	5.00	3.65	1.35	5.80	25.80	3.60

The gastric juice employed in this experiment was obtained from a dog with a gastric fistula and esophagotomy, in response to continuous sham-feeding with raw minced meat. The secretion, collected during a forty-minute period, was immediately filtered and used for the above experiment. The total acidity and the free acidity were respectively 158 and 144 m.eq. per liter. The total nitrogen was 29.40 mg. per cent. The nonprotein nitrogen, as determined by the acetone method and by precipitation with 7.5 per cent trichloracetic acid at 80° C., was found to be 16.2 mg. per cent. The samples, treated as indicated in the table, were heated simultaneously in a boiling water-bath for five minutes, cooled simultaneously in a cold water-bath, and filtered through Whatman's filter paper No. 42. pH values were only roughly determined by means of B.D.H. capillator colorimeter.

It is evident that, with the progressive lowering of the acidity of the gastric juice by the preliminary addition of sodium hydroxide, less and less protein material is coagulated by heating, until at a pH above 5.8 no precipitation takes place whatsoever.

In the filtrates obtained by the addition of trichloracetic acid to fresh gastric juice, secreted in response to sham-feeding, and subsequent heating to 80°, the biuret reaction is negative, but with "histamine" gastric juice the filtrates always show a blue color. In this connection it should be mentioned that the main bulk of protein material in the gastric secretion elicited by sham-feeding consists of the protein described by Webster and Komarov (1932) as a mucoprotein of the gastric secretion. This mucoprotein has a nitrogen content of about 14.0 per cent. It was found recently by the writer (unpublished data) that the main bulk of the protein of the "histamine" gastric secretion consists of an entirely different protein, containing a lower percentage (i.e. 12 per cent) of nitrogen. This protein has also the more pronounced characteristics of a mucin, since after preliminary acid hydrolysis it shows a very high reducing power equivalent to a 30 to 33 per cent glucose content. Apparently the former protein is precipitated completely by trichloracetic acid, whereas for the latter precipitation is not complete. This problem is not yet entirely solved; further study of it is now in progress.

When trichloracetic acid is added directly to gastric juice of normal acidity (about 0.15 normal) in an amount of from 5 to 10 per cent, no pre-

cipitation takes place within twenty-four hours, if the mixture is kept at a temperature not exceeding 20° C. However, at temperatures above 24° C. precipitation of protein occurs, and at 38° C. the proteins of the gastric juice are completely precipitated by trichloracetic acid within two or three hours. Special experiments were performed which showed that under such conditions gastric juice in the presence of 7.5 per cent trichloracetic acid exhibits considerable digestive power. These observations strongly suggest that the protein material present in freshly secreted gastric juice is not precipitable by means of trichloracetic acid at room temperature, but that the proteins of the gastric juice acquire this property at a certain stage of hydrolysis. Probably that is also the explanation of another fact observed in the course of this study, namely, that, when trichloracetic acid is added to previously neutralized gastric juice, it always causes some precipitation of protein material at 20° C.; the precipitation, however, is never complete, even when supplemented by heating to 80° C.

The modified form of Somogyi's method which we have employed likewise yielded very consistent results. With freshly secreted gastric juice, the values for nonprotein nitrogen were about 10 per cent lower, and with autolyzed gastric juice they were about 20 per cent lower than those obtained by the acetone method.

The Folin-Wu method, in the various modifications used, was found to be entirely unreliable. At pH higher than 3.5, tungstic acid does not produce any precipitation. With the increase of the acidity more and more nitrogen is precipitated until, at pH less than 1.0, the amount of nitrogen precipitated very closely approximates the amount precipitated by phosphotungstic acid.

Table I (samples 3a, 3b, 3c, 4a, and 4b) shows that the most rigid precautions should be taken to prevent autodigestion of the gastric juice, if it is desired to obtain true values for nonprotein nitrogen, since a considerable increase in nonprotein nitrogen occurs within twenty-four hours even at room temperature, whereas at 38° C. from 66 to 75 per cent of the protein originally present is hydrolyzed.

The analysis of sample 10 demonstrated that even a very slight, almost imperceptible, contamination of the gastric juice by an exudate from the ulcerated skin around the fistula may lead to an entirely wrong conception regarding the composition of the gastric juice. It is evident that the absolute figures for the various fractions of nitrogen in sample 10 do not admit of comparison with the corresponding figures for really pure gastric juice. In contrast to the results obtained with pure gastric juice, tungstic acid caused considerable precipitation at pH 6 or pH 3.5. This result was apparently due to the presence of blood proteins in various stages of hydrolysis.

From the experimental data presented in this article certain conclusions may be drawn with regard to the composition of gastric juice, as affected by the nature of the stimulus producing it. The gastric secretion elicited through the vagal mechanism (sham-feeding juice and psychic secretion) differs strikingly in composition from "histamine" juice, especially in regard to its

content of protein nitrogen. The greatest concentration of protein nitrogen was observed in the sham-feeding juice, which represents the glandular response to nervous stimulation in its most intense form. The protein nitrogen content is considerably lower in the psychic secretion, and still lower in the typical histamine secretion (samples 5, 6, and 7). When psychic impulses are not entirely excluded, the protein nitrogen content of the histamine juice approximates that of the psychic secretion (samples 8 and 9). Nonprotein nitrogen forms a considerable part of the total nitrogen of gastric juice under all conditions of stimulation. In the vagus juice it constitutes from 36.5 to 44 per cent of the total nitrogen; in the typical histamine juice the proportion is much higher, viz., from 67 to 78 per cent. The absolute figures for nonprotein nitrogen vary from 12.04 mg. per cent in the sham-feeding juice to 6.02 mg. per cent in the psychic secretion, and from 9.52 to 5.88 mg. per cent in the histamine juice.

From the experience gained in this laboratory over a number of years, it appears that the variations both in protein and nonprotein nitrogen shown in Table I cover fairly well the range of variations which may be observed in the secretion of the whole stomach in normal dogs under experimental conditions. The values for nonprotein nitrogen in the gastric contents of human beings, as reported in the literature, are in most cases much higher. Polland, Roberts, and Bloomfield reported 40 to 80 mg. per cent of nonprotein nitrogen, Martin 20 to 48 mg. per cent, Mielke 28.4 to 85.5 mg. per cent, and Diehl 27.1 to 86.8 mg. per cent. It should be noted that these data were obtained by the Folin-Wu method in its original, or in a somewhat modified form, or by trichloracetic acid precipitation at room temperature. On the other hand, our figures for nonprotein nitrogen are fairly close to those reported by Baltzer (1934), which were obtained by the ultrafiltration method, or by means of precipitation with ferric hydrosol.

The partition of nonprotein nitrogen was studied in the acetone filtrates obtained from two large samples (4 and 6 liters, respectively) of gastric juice secreted in response to sham-feeding, as shown in Table II. Urea, ammonia, and other volatile bases, and creatine constitute about 20 per cent of the nonprotein nitrogen. About 37 per cent of the nonprotein nitrogen is represented by the physiologically important fraction of nitrogenous bases precipitable by phosphotungstic acid (volatile bases excluded). The amount of nitrogen precipitated by silver nitrate at pH 2.8 and pH 7.0—"purine" and "histidine" fractions—is quite negligible. Almost the whole bulk of the nitrogenous bases is present in the "arginine" fraction precipitable by silver nitrate and barium hydroxide at pH 8.0 to 9.0 and in the filtrate of the former—"lysine" fraction. This observation is of some interest, in view of the previously described experiments, which showed that the arginine fraction exerts a histamine-like action on blood pressure and also on gastric, pancreatic, and biliary secretion, and that the lysine fraction gives a positive Sakaguchi reaction and shows a guanidine-like action on skeletal muscles, blood pressure, and on pancreatic and biliary secretion (Komarov, 1935).

SUMMARY

1. Three methods for the determination of nonprotein nitrogen in gastric juice were found to give uniform and comparable results: heat coagulation, precipitation with two volumes of acetone, and precipitation with trichloracetic acid at 80° C. The last two methods were found to be particularly useful for routine work.

2. In regard to the partition of nitrogen there was a striking difference between the gastric secretion elicited by the vagal mechanism (sham-feeding) and that provoked by histamine.

3. A more detailed study was made of the partition of nitrogen in gastric juice secreted in response to sham-feeding, which among other data revealed the fact that a surprisingly large proportion of nonprotein nitrogen (about 40 per cent) is formed by the physiologically important fraction of nitrogenous bases.

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STILL'S DISEASE WITH HYPERGLOBULINEMIA

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IN 1897, Still[1] described a form of arthritis in children which has since then been called by his name. The syndrome was characterized by a mild nondestructive arthritis, a swelling of the lymph glands, enlargement of the spleen and fever. The arthritis was multiple and usually recurrent. The lymphadenopathy was general; the glands, while firm, never exceeded the size of a nut. The splenomegaly, sometimes minimal, was occasionally marked, the spleen in these cases extending below the level of the navel. The fever was never high and was sometimes continuous, sometimes recurrent; it bore no relation to the severity of the arthritis. Of his 12 cases 3 came to autopsy; all three showed an adhesive pericarditis that had not been diagnosed before death.

A year earlier Chauffard and Ramond[2] had described 7 cases of a similar syndrome but without the splenomegaly. Moreover, their patients were all adults, ranging in age from 22 to 61 years. Since then, especially in France, the condition is often called the Chauffard-Still syndrome.

During the past thirty years a considerable number of cases of Still's disease have been described, chiefly in England and Germany, more recently in this country and in France. A good analysis of the cases reported up to 1930 may be found in the article of Chevallier.[3] The disease may occur at any age, but is most characteristically a disease of the young; one-third of the cases have been found in childhood, one-third during adolescence, and one-third beyond the age of 25 years, the oldest reported being 61 years old.[3, 4] While a nondestructive periarthritis is an essential feature of the disease, this symptom may vary from an extreme form producing complete crippling to a relatively mild involvement with intermissions. The adenopathy, while uniformly present, varies greatly in extent; the glands are never very large and in general the degree of adenopathy varies with the intensity of the joint involvement. The splenomegaly also varies, being occasionally absent. Usually the spleen can be felt a few fingerbreadths below the costal margin and is sometimes enormous, extending below the navel. Fever is rarely absent and while sometimes inconsiderable, may run a high septic course for weeks at a time. There is always progressive emaciation and a marked secondary anemia. The white count usually shows a moderate leucocytosis early in the disease; later a leucopenia, sometimes extreme,[5] is the rule. Of the complications, pericarditis is the most frequent. It is usually fibrous, leading to obliteration of the pericardial sac, but may be exudative. Pleuritic adhesions are constant.

The autopsy findings usually show no very characteristic changes. The joints show periarticular thickening, the synovial membrane being usually not

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involved. The lymph glands show only indurative processes in the interior, no specific changes.[6] The splenic changes are limited to a hyperplasia of the cellular elements and a moderate congestion; there is no fibrosis.[4]

It is obvious that Still's disease, as extended by later observers, is not a sharply defined symptom complex. If applied to adults it certainly includes cases of Felty's syndrome[7] which both clinically and pathologically are indistinguishable from Still's disease. Cases in which the arthritis is minimal but in which fever, leucopenia, and splenomegaly are marked would suggest Banti's disease; if the peritoneum were involved, as the pericardium and pleura so often are, the two diseases would be nearly indistinguishable. However, I do not know of any case of Still's disease with ascites, and the liver is enlarged only if there is a passive congestion due to heart failure or if the organ is the site of amyloid degeneration.[8] Moreover, since moderate adenopathy and slight splenic enlargement are not uncommon in children suffering from any infection, it is difficult to draw the line between ordinary cases of juvenile polyarthritis and some cases of Still's disease. Smith and Gallup[9] go so far as to say: "This condition was formerly spoken of as 'Still's Disease,' but the name has now been generally discarded and the condition is recognized merely as a modification of atrophic arthritis."

Whatever one may conclude regarding the justification of considering the Still-Chauffard syndrome as a clinical entity, it will remain a useful means for gathering together that group of cases, no longer rare, that show a periarthritis, splenomegaly, adenopathy, fever, anemia, and usually involvement of pericardium and pleura and in which all the more usual diagnoses can pretty definitely be excluded. It is for this reason that the two cases reported here, certainly not typical of the disease as described by Still, have been included within this group.

Within the last few years increasing attention has been directed to the visceral manifestations of lupus erythematosus disseminatus. These consist of a long-continued fever, arthritis, pleurisy, pericarditis, pneumonitis, nephritis, and leucopenia. Disseminated cerebral manifestations may occur and in all of our cases that were observed for a sufficient length of time, high blood serum globulin and euglobulin values, quite comparable to those reported below, were found. The suspicion is justified that cases of disseminated lupus erythematosus without skin manifestations may not be uncommon, and that lupus, the Libman-Sacks endocarditis, the Still-Chauffard syndrome, and Felty's disease may all be manifestations of the same disorder. The final solution of this question must await the discovery of a common etiologic agent, if any.

The technique of blood protein determinations used at the laboratory of the Jewish Hospital of St. Louis closely follows that of Howe,[10] except that serum is used instead of plasma. This method in skillful hands gives trustworthy results and is rivaled only by refractometric methods and possibly by the new one of Campbell and Hanna.[11]

Different observers have varied somewhat widely in their figures of normal percentages of albumen and globulin in plasma and serum. This variation may

in part be due to differences in technique but may also be due to a variation in the clinical material used. Peters and Eisenman[12] give as their normal values: total serum protein 6 to 8 per cent, serum albumen 4 to 5 per cent, and serum globulin 1.4 to 3 per cent. Other observers vary in either direction. The suggestion of Starlinger and Winands[13] that only values of serum globulin greater than 4.0 per cent be considered indicative of hyperglobulinemia probably deserves acceptance.

Serum globulin itself appears to be a mixture of proteins and of these euglobulin is apparently the one that has clinical significance. The highest normal value found by Ling[14] was 0.333 per cent, and it is safe to say that, if the determinations are made by Howe's method, any euglobulin value above 0.5 per cent is unusual and anything over 0.75 per cent is definitely pathologic. In the cases reported here much higher figures were found.

Convenient qualitative indications of an excess of euglobulin are furnished by the dilution test and the formol-gel test. The former is done by adding 9 c.c. distilled water to 1 c.c. serum. A cloudy solution in which upon standing a flocculent precipitate forms indicates an excess of euglobulin. In the formol-gel test one drop of 30 per cent formalin is added to 1 c.c. clear serum in a small test tube and mixed by gentle shaking. In the presence of a considerable excess of euglobulin the mixture gels and becomes opaque within a few hours, sometimes within a few minutes.

Infectious diseases are usually accompanied by a rise in the percentage of serum globulin and often by a fall in serum albumen. Usually these variations are relatively slight, so that there is no inversion of the albumen-globulin ratio. In certain diseases, however, the rise in the globulin value may be so great that it not only exceeds the critical 4.0 per cent but may be relatively enormous so that, in spite of a considerable fall in the percentage of serum albumen, the amount of total serum protein far exceeds the normal.

Kala azar was the first infection in which extraordinarily high values of serum globulin were observed and a marked hyperglobulinemia is apparently a constant finding in this disease. Wu's case[15] in which the total protein values were 10.52 per cent with 7.06 per cent globulin apparently represents a record in this disease. Ordinarily the serum globulin does not exceed 5.0 per cent. The euglobulin values are usually between 2 per cent and 3 per cent.

In syphilis, especially in the florid secondary cases, there is often a fall in the serum albumen and a corresponding rise in serum globulin. The latter is usually moderate[16] but may reach 5.0 per cent and at least once[17] has been reported as 6.5 per cent.

The highest globulin values ever found in blood serum have occurred in multiple myeloma. Thus, in one case reported by Shirer, Duncan, and Haden,[18] the serum protein amounted to 13.78 per cent and the globulin to 11.34 per cent. In all cases of multiple myeloma with hyperproteinemia the high protein values have been due to a substance supposed to be globulin.

Just what this globulin-like substance is remains uncertain. It is salted out of the serum with the euglobulin, and some observers[19] believe it definitely to be euglobulin. Others, especially Shirer, Duncan, and Haden[18] and Cantarow,[20] be-

lieve it to be the Bence-Jones protein that is so often found in the urine in this disease. Our own very limited experience with this substance indicates that it is neither euglobulin nor Bence-Jones protein, and perhaps not even a true globulin.

Within the past few years two cases were observed, one at the Jewish Hospital and one at Barnes Hospital of St. Louis, which fall more readily into the syndrome of Still's disease, in its more inclusive form, than into any other category, and whose serum globulin and euglobulin values reached a level hitherto observed only in kala azar and possibly in multiple myeloma.

CASE 1.—L. S., 18 years old, though looking like a child of 12, entered Barnes Hospital in February, 1935. For over a year he had had a migrating polyarthritis and this had left behind a swelling of the elbows and knees, exaggerated by the muscular atrophy of the arms and legs. There was some limitation of motion. On admission his emaciation and pallor were extreme and there was evidence of pericardial effusion. The spleen was palpable. The liver was enlarged and tender, suggesting chronic passive congestion. There was a generalized lymphadenopathy, but the glands were not very large. During the three months he was in hospital he ran an irregular fever, sometimes exceeding 104°; the temperature was never normal for more than twenty-four hours at a time. The arthritis flared up from time to time, but there were intervals during which he was quite free from pain. His blood showed a marked secondary anemia, requiring repeated blood transfusions. The white cells varied from a slight leucocytosis (12,250) to a moderate leucopenia (5,100); the hemogram constantly showed a shift to the left. The Kahn test was negative; the serum calcium was 9.3 mg. per cent and serum phosphorus 4.5 per cent. Neither blood serum nor urine contained any Bence-Jones protein.

The pericardium was aspirated and 35 c.c. clear fluid were withdrawn; culture and guinea pig inoculation were negative. Repeated blood cultures were negative as were also all the usual agglutination tests.

He remained in hospital until May 17, the clinical picture remaining unchanged and the fever persistent. After a few months at home, he reentered in August on account of dyspnea and thoracic pain. There was again evidence of pericardial effusion and 110 c.c. turbid fluid were withdrawn. The cells consisted chiefly of neutrophiles with many monocytes and rare eosinophiles; culture of the fluid showed no growth. The anemia was again extreme with a more marked leucopenia (4600) and a normal hemogram. The spleen was larger than before, the liver also somewhat enlarged. The Congo red test for amyloidosis was negative.

On October 2, he insisted upon leaving for his home in the country, where he died two days later. No autopsy could be obtained.

The blood serum had always shown marked flocculation on dilution with distilled water; the formol-gel test always produced milkiness and jelling within five minutes. The serum protein values were as follows:

DATE	TOTAL PROTEIN	SERUM ALBUMEN	SERUM GLOBULIN	EUGLOBULIN	PSEUDO-GLOBULIN
3/ 2/35	9.71 per cent	2.38 per cent	7.33 per cent	2.59 per cent	4.74 per cent
4/17/35	14.1	2.4	11.7		
4/29/35	11.4	2.3	9.1		
5/18/35	10.36	2.14	8.22	3.67	4.55
8/ 8/35	8.4	1.4	7.0		
9/12/35	7.25	1.76	5.59	1.67	3.92

CASE 2.—F. R., aged 59, entered the Jewish Hospital of St. Louis in September, 1934, complaining of loss of weight and increasing weakness. During the previous summer she had suffered much from arthritis, involving chiefly the left shoulder and elbow. The joints were

painful and showed swelling, redness, tenderness, and limitation of motion. When she entered the hospital these manifestations had pretty well subsided, but there was still some thickening about the joints and some arthritis of the cervical spine. She was found to be running an irregular fever, at first only occasionally reaching 102°, but later during her stay in hospital exceeding this level nearly every evening. Physical examination showed a systolic cardiac murmur that had been present at a previous admission nine years before, a moderate cervical, axillary and inguinal adenopathy, a slightly enlarged liver, and a greatly enlarged spleen. The blood count on admission showed: red cells 4,400,000; hemoglobin 90 per cent; white cells 3,900; hemogram:

Ba	Eo	My	Ju	St	Se	Ly	Mo
1	1	0	0	17	50	27	4

During her three months' stay in hospital the white count was never quite so low again but exceeded 6,500 only once, while a moderate secondary anemia made its appearance. Repeated blood cultures were negative. The Kahn test was sometimes positive, sometimes negative; the Wassermann, when not anticomplementary, was negative; there was nothing in the history suggesting syphilis. All the routine agglutination tests were negative, as were intradermal tests for tuberculosis and brucellosis. Splenic puncture and sternal marrow biopsy gave normal findings. The urine, at first normal, later contained albumen, casts, and red cells. Repeated examination of blood and urine was negative for Bence-Jones protein.

In January, 1935, the arthritis, which had not been marked for several months, flared up again with pain, tenderness, redness, and swelling of the right ankle. It subsided after some weeks, but recurred again two months later.

During all of this time until her death in May she continued to run a high daily fever of a septic type. The blood picture showed a secondary anemia, progressively more severe, and a moderate leucopenia. A typical count was that of March 13, 1935: red cells 2,810,000; hemoglobin 65 per cent; white cells 7,500; hemogram:

Ba	Eo	My	Ju	St	Se	Ly	Mo
0	2	0	0	6	34	56	2

The platelet count was 180,000; toward the end of her illness many small petechiae were seen scattered over the entire body. Repeated blood cultures were negative. The spleen slowly grew in size, being firm and smooth, and finally extending some 11 cm. below the costal margin. The very moderate adenopathy remained unchanged. The liver was always distinctly palpable. There was never any free fluid in the abdomen.

In May, 1935, seven months after entering the hospital, she died in coma with a terminal pneumonia. Unfortunately, autopsy was refused.

The blood serum, like that of the first case, showed a heavy flocculation upon dilution with distilled water, indicating the presence of a large excess of euglobulin. All sera except the last solidified and became opaque promptly upon the addition of formol. The serum protein values were as follows:

DATE	TOTAL PROTEIN	SERUM ALBUMEN	SERUM GLOBULIN	EUGLOBULIN	PSEUDO-GLOBULIN
11/ 2/34	7.7 per cent	3.2 per cent	4.5 per cent	2.3 per cent	2.2 per cent
11/ 7/34	9.0	3.9	5.1	1.2	3.9
12/ 4/34	7.1	3.3	3.8	1.1	2.7
3/16/35	7.87	2.10	5.77	2.24	3.53
5/21/35	8.19	3.90	4.29	0.83	3.46

COMMENT

There may well be a difference of opinion regarding the propriety of considering these two cases as examples of Still's disease, if only because the delimitation of this syndrome is itself somewhat vague. In both cases Banti's

disease was considered, but the continuous high fever, the arthritis, the adenopathy and the total absence of ascites did not fit this picture. A complete x-ray study of the bones should have been done to exclude multiple myeloma, but nothing in the clinical picture suggested this diagnosis, nor did blood or urine ever contain Bence-Jones protein. The two cases seem worth reporting because it may be that a study of the blood proteins may help us to a further knowledge of those very interesting cases that have been grouped together under the name of Chauffard-Still syndrome.

SUMMARY

Two cases are reported characterized by arthritis, adenopathy, splenomegaly, and long-continued high fever. For want of a better diagnosis they are included in the so-called Chauffard-Still syndrome.

Both cases show hyperglobulinemia, with an extremely high euglobulin percentage.

I desire to express my thanks to Dr. David P. Barr, Head of the Department of Medicine of Washington University, for permission to report the first case; and to Dr. Michael Somogyi, biochemist at the Jewish Hospital of St. Louis, for the blood protein determinations.

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THE INFLUENCE OF CALCIUM SALTS ON DIGITALIS ACTION*

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IT IS held quite widely by clinicians and research workers that intravenous calcium medication is dangerous, especially when strong concentrations are used, and exceptionally so in digitalized patients. The intravenous use of calcium salts is extensive. It has been used in the treatment of lead poisoning; after parathyroidectomy; in tetany; tuberculosis; nephritis with edema; after hemorrhage; in heart disease; in allergic diseases, such as eczema, urticaria, angioneurotic edema, asthma, hay fever, etc.; deficient calcification of the bones; to relieve the pain in inoperable cancer; in alkalosis; in jaundice preliminary to operations, etc.

Bower and Mengle[1] report two deaths in patients following the intramuscular administration of digitalis and the intravenous injection of 10 c.c. of 10 per cent calcium gluconate in one case, and 10 c.c. of 10 per cent calcium chloride in another. Lloyd[2] reports that rapid intravenous injection of calcium chloride (10 per cent) is dangerous; the electrocardiogram showing what he interpreted as sino-auricular block. Berliner[3] found that the intravenous injection of 10 c.c. 20 per cent calcium gluconate in man produced electrocardiogram changes, which indicated damage to the heart. He states that when injected slowly and in proper amounts, calcium salts are harmless, but when administered rapidly they are potentially dangerous. Lieberman,[4] working with dogs, found that when calcium gluconate is given slowly (about 0.5 c.c. per minute of about 1.2 per cent) about ten times the amount could be injected as if the rate of injection was 1 c.c. per minute.

Calcium salts injected into the blood stream affect the heart somewhat like digitalis. Gold and Edwards[5] found a synergism between calcium and ouabain in dogs. Lieberman found only an additive effect of calcium with digitalis and with scillaren B. Nyiri and DuBois,[6] working with frogs, found that calcium, above a certain concentration, intensifies the action of digitalis. Weichmann[7] also found that in the isolated frog's heart calcium ions increase the action of digitalis. Loewi[8] accepts the synergistic action of calcium and digitalis and explains the synergism by stating that digitalis sensitizes the heart toward calcium ions. Combating the explanation of Loewi, Forscher,[9] also Fischer,[10] claims that digitalis exerts a specific action independent of the calcium. Fischer offers the following explanation: The heart is sensitized by digitoxin for certain kinds of stimuli, among others for calcium ions, and there is a reciprocal synergism between calcium and digitalis. Schuntermann[11]

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thinks the synergism is due to the condition and amount of electrolytes in the tissues (electrolyte milieu).

Some of the experiments upon which the above opinions are based were on the isolated heart. Some were on intact animals. Each method has its advantages and disadvantages, but it would seem more physiologic to use intact animals, and for comparison with man, warm-blooded animals would seem preferable. Colldahl[12] used intact tadpoles, and, to insure better absorption, treated them with the glucoside cyclamin, after the method of Overton. By this method a definite constant concentration of the drug may be kept in the blood. This method has the advantage that a known concentration of calcium as well as digitoxin can be kept in the circulation. Colldahl found that digitalis or increased calcium content of the blood caused a decrease in the heart rate. A digitoxin concentration of 1:800,000 had an action similar to 1:500 of calcium chloride. Quite contrary to most of the reported work, however, he found that calcium lessens the toxic action of digitoxin. He states that his results correspond with certain clinical observations of which he quotes the following:

Billigheimer[13] thinks that the action of digitoxin on the heart is due to the liberation of calcium ions, and states that cases of tetany (calcium deficiency) with sound heart, tolerate little digitalis. On the other hand, cases of encephalitis in which the blood calcium content is quite high tolerate enormous doses of digitalis.

Singer[14] says that "while calcium increases and hastens the action of digitalis on the heart, it lessens the untoward actions of digitalis on the parasympathetic system. It is both the whip and the rein for digitalis." Seelig[15] calls attention to the possible ill effects on the heart by 10 per cent calcium chloride intravenously, and recommends that 1 per cent be used and injected slowly. This suggestion corresponds to the findings of Lloyd, Lieberman, and to our own, and we believe it has not received the attention it merits.

Quite recently Nahum and Hoff[16] have studied the effect of calcium on the digitalized heart. They used rabbits in their work and studied particularly the electrocardiogram changes produced. They found that digitalized rabbits tolerated as much calcium as controls, and concluded that in the normal nonanesthetized rabbit heart the effects of calcium and digitalis are not additive. Their work is not quite comparable with ours since after "arrest" of the heart with calcium in controls, they were able to use the animals again with digitalis. In clinical cases where death occurred after digitalis and calcium, they incline to the opinion that it was entirely due to digitalis. We think it was more likely due to the too rapid injection of the calcium salts.

The work of Nahum and Hoff has been criticized adversely by Gold and Kwit[17] on the fact that the dose of digitalis as used by Nahum and Hoff, and referred to as 50 to 75 per cent of the calculated lethal dose, was only about 6 to 9 per cent of the lethal dose for the rabbit, the lethal dose of digitalis for the rabbit being about 8 times as much as for the cat.

EXPERIMENTS

Dogs were used in all our experiments. They were anesthetized with pentobarbital sodium, 35 mg. per kg. body weight, given intraperitoneally. This drug as given affects the heart but slightly, and, therefore, is well suited to study drugs that kill by affecting the heart. Injection of the calcium salts was into the femoral vein, each injection, unless specified, being made in about one minute, and injections were repeated at five-minute intervals.

FATAL DOSE OF CALCIUM CHLORIDE

WT. OF DOG IN KG.	PER CENT $CaCl_2$	INJECTIONS (C.C.) EVERY 5 MIN.	TOTAL DOSE C.C.	FATAL DOSE $CaCl_2$ PER KG.
10.9	3.7	10.0	360	1.222
8.6	5.0	15.0	150	0.880
6.0	5.0	10.0	80	0.666
25.0	5.0	12.5	275	0.550
23.2	5.0	11.6	442	0.960
15.0	5.0	10.0	280	0.933
13.6	5.0	20.0	80	0.300
9.5	10.0	10.0 slowly	60	0.630
20.9	10.0	10.0 very slowly	160	0.765

From the above experiments it is concluded that the amount of calcium that may be injected before death occurs depends largely on the rate of injection. Twenty cubic centimeters of 5 per cent injected rapidly may kill at one injection, whereas 400 c.c., or over one gram per kilogram, may be given if injected slowly.

FATAL DOSE OF CALCIUM GLUCONATE

WT. OF DOG IN KG.	% Ca GLUCONATE IN SALINE	RATE (C.C.) EVERY 5 MIN.	TOTAL (C.C.)	GM.	EQ. TO $cacl_2$ PER KG.
8.6	10	50	250	3.08	0.740
13.2	10	10	450	3.00	0.740

While these large doses may be injected, three injections of 20 c.c. each killed a 10 kg. dog, and one such injection may kill a smaller animal. Two or 3 per cent solutions of calcium gluconate may be injected ad libitum and kill mainly by volume effect.

FATAL DOSE OF DIGITALIS

In dogs anesthetized as we have done, with pentobarbital, 35 mg. per kg., the fatal dose of the digitalis was 1 c.c. of the tincture per kg. In fact the dose is so uniform that we think that this method may be used to standardize digitalis. The variation we have found in a number of healthy dogs is not over 10 per cent from this figure. The number of animals used in this case,[9] while sufficient for the present purpose, is not enough to say that the method may be used for standardization. We are at work on this problem.

EXPERIMENTS WITH DIGITALIS ALONE

A strength corresponding to the 10 per cent tincture in 25 per cent alcohol was used, and 1 c.c. injected into the femoral vein every five minutes.

WT. OF DOG IN KG.	RATE (C.C.) EVERY 5 MIN.	FATAL DOSE (C.C.) 10% TINCTURE
7.3	1.0	8
6.0	1.0	6
12.0	1.0	11
8.2	1.0	8
7.7	2.0	8
16.6	2.0	18
14.6	2.0	16
13.6	1.0	13
10.9	1.0	10

DIGITALIS GIVEN FIRST, 1 C.C. EVERY FIVE MINUTES, FOLLOWED BY CALCIUM GLUCONATE

WT. OF DOG IN KG.	DIGITALIS (C.C.) 10%	Ca GLUCONATE (C.C.) 10%	FATAL DOSE (%) DIGITALIS	FATAL DOSE (%) Ca GLUCONATE
10.0	6	120	60	40
6.6	4	100	52	46
13.0	12	30	92	8
	Calcium Gluconate Given Before Digitalis			
10.0	9	60	90	20
8.2	8	6	97	2
	Calcium Gluconate (2%), 1 c.c. Injections, Alternated with Digitalis			
13.0	11	11	90	0.4
10.0	11	11	110	0.6

In another dog, 8 kg., rapid injection of 25 c.c. 10 per cent calcium gluconate, after 50 per cent of the fatal dose of digitalis, killed almost immediately. Slow injection causes no such effect. These experiments show that calcium gluconate in larger or smaller dose has no perceptible action on digitalis, other than an additive toxic effect.

DIGITALIS WITH CALCIUM CHLORIDE

Since the toxic dose of calcium chloride varies with the rate of injection, we have tried to give the injections at a uniform rate. The toxic dose of calcium chloride for purpose of calculation is taken at 550 mg. per kg. body weight. This was the minimum fatal dose found when we injected 5 per cent calcium chloride at a rate of 0.5 c.c. per kg. every five minutes. If injected more slowly, this figure may not be correct, but it will serve for purposes of comparison, and if any striking effect of giving digitalis and calcium chloride together is an actuality, it should demonstrate it. Because it has been stated by some that digitalization is sometimes rendered less toxic by calcium chloride, we have varied the doses and concentrations of the calcium.

EFFECT OF SUDDEN CHANGES OF CALCIUM CONCENTRATION ON THE HEART

Carl Schmidt (quoted from Mathews' *Physiological Chemistry*) gives the calcium concentration of the blood at 0.0193 per cent calcium phosphate. This is equivalent to 0.007 per cent calcium. Since the volume of blood in a person weighing 70 kg. is about 5,500 c.c., the total amount of calcium would be 0.385 grams.

DIGITALIS ALTERNATED WITH $CaCl_2$
DIGITALIS WITH 0.5% $CaCl_2$, ALTERNATE INJECTIONS OF 1 C.C. DIGITALIS, 0.5 C.C. $CaCl_2$

WT. OF DOG IN KG.	DIGITALIS	$CaCl_2$	FATAL DOSE (%) DIGITALIS	$CaCl_2$
8.4	9.0	4	107	0.2
Digitalis with 1% $CaCl_2$, Alternate Injections				
8.0	9.0	9	110	0.5
Digitalis Given First, Followed by 5% Calcium Chloride 0.5 c.c. per kg.				
18.2	9.1	118	50	55.0
20.0	10.0	190	50	44.0
26.0	14.0	60	54	20.0
The rapid injection in the last case (15 c.c. at dose) was a factor in death.				
17.3	9.0	81	53	43.0
Alternating Doses of Digitalis and 5% Calcium Chloride				
20.9	13.0	84	62	44.0

Each 10 c.c. of a 10 per cent solution of calcium chloride contains 0.36 gm. of calcium. Consequently the intravenous injection of 10 c.c. would double the normal calcium concentration immediately. Since this may reach the heart in several times this concentration, it may cause immediate stoppage. The effect is directly on the muscle, since we have found it is not prevented by atropine. The effect, however, on the tracing is similar to that which occurs after stimulation of the vagus. Rapid injection of many drugs may cause a stoppage of the heart, which may be temporary or permanent, and for lack of knowledge of the detailed mechanism is described as a "shock" effect; in this case it is apparently due to sudden change of the electrolyte milieu. Such reactions emphasize the importance of slow injections of any intravenous medicament. In the case of calcium chloride, a 10 c.c. dose should occupy at least two minutes, at a uniform rate.

A recent study by Hoff and Nahum,[18] using the electrocardiogram, shows that 1 to 2 c.c. of a 10 per cent calcium chloride solution injected at the rate of 2 c.c. per minute in unanesthetized rabbits produces a slowing of the heart, auriculoventricular delay, and auricular fibrillation. Larger doses cause extrasystoles from many foci, periodic cardiac arrest alternating with ectopic ventricular beats (Luciani periods), and cardiac arrest.

From the above experiments the following conclusions may be drawn:

1. Calcium salts affect the heart in a manner similar to digitalis, and their action is additive.

2. Calcium salts may be injected intravenously in quite large amounts if given slowly. The rapid injection of 5 per cent is potentially dangerous, either in presence or absence of digitalization.

3. There is no specific danger in the slow administration of calcium after digitalis other than an additive effect.

4. Calcium gluconate is safer than calcium chloride for intravenous use only because it contains less calcium. A saturated water solution (about 3 per cent) may be injected at the rate of 10 c.c. per minute without much danger. One-half this rate, however, is recommended.

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LABORATORY METHODS

THE MICRODETERMINATION OF CALCIUM IN WATER*

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FROM time to time most laboratories are called upon to determine the calcium content of water, or they may desire to check upon the efficiency of the operation of a water softener or still. Since the methods given in *Standard Methods of Water Analysis*[1] require considerable time, it occurred to us that the Clark-Collip modification of the Kramer-Tisdall method might be employed for this purpose. This method is commonly used for the determination of calcium in blood serum, but, as far as we know, it has never been suggested for the determination of calcium in water.

METHOD

The following technique (as given by Mattice[2]) was employed: 5 ml. of water and 1 ml. of saturated ammonium oxalate are placed in a conical (15 ml.) centrifuge tube. The contents are mixed by gentle agitation and allowed to stand for two to four hours (Mattice[2] recommends that, when convenient, they be allowed to stand overnight in the icebox). At the end of the period, the tubes are centrifuged and the precipitate is washed twice with dilute ammonia, then dissolved in 2 ml. of approximately N H_2SO_4, and the solution after heating is titrated with 0.01 N $KMnO_4$ in the usual manner. A blank determination is conducted by adding 1 ml. of the ammonium oxalate solution to 5 ml. of distilled water.

RESULTS

In order to try out the method, a stock calcium solution was prepared and standardized according to *Standard Methods of Water Analysis*. The solution, when diluted, contained 0.09 mg. calcium per ml. This was added to distilled water in varying amounts, and the calcium was determined by the method given above. The results are given in Table I. Each figure given is the average of closely agreeing duplicates, determinations made upon different days. In every case, the "blank" has been deducted from the total amount of $KMnO_4$ used in the titrations.

*From the Arizona State Laboratory, Tucson.
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TABLE I

RECOVERY OF CALCIUM ADDED TO DISTILLED WATER

1 ML. CA SOLUTION = 0.09 MG. CA

ML. Ca SOLUTION	ML. DISTILLED H_2O	MG. Ca ADDED	ML. 0.01 $KMnO_4$	MG. Ca RECOVERED
1	4	0.09	0.45	0.09
2	3	0.18	0.90	0.18
3	2	0.27	1.40	0.28
4	1	0.36	1.80	0.36
5	0	0.45	2.25	0.45

Table I shows that excellent recoveries were obtained, so the experiment was repeated by adding a calcium solution of known strength to tap water. Many analyses of the tap water according to *Standard Methods of Water Analysis*[1] have shown that it varies between 40 to 45 parts per million of calcium with an average of about 42 parts per million.

TABLE II

RECOVERED OF Ca ADDED TO TAP WATER

1 ML. CA SOLUTION = 0.09 MG. CA

ML. Ca SOLUTION	ML. TAP WATER	MG. Ca PRESENT	ML. 0.01 N $KMnO_4$	MG. Ca RECOVERED
0	5	0.21	1.05	0.210
1	4	0.258	1.25	0.250
2	3	0.306	1.55	0.310
3	2	0.354	1.80	0.360
4	1	0.402	2.05	0.410
5	0	0.450	2.30	0.460

The recoveries obtained agree well with the amounts of Calcium known to be present. In order to determine how sensitive the method is a more dilute solution of Calcium was added to distilled water. The results are given in Table III.

TABLE III

RECOVERY OF SMALL AMOUNTS OF CALCIUM ADDED TO DISTILLED WATER

1 ML. CA SOLUTION = 0.01 MG. CA

ML. Ca SOLUTION	ML. DISTILLED H_2O	MG. Ca PRESENT	ML. 0.01 N $KMnO_4$	MG. Ca RECOVERED
1	4	0.01	0.05	0.01
2	3	0.02	0.10	0.02
3	2	0.03	0.15	0.03
4	1	0.04	0.2	0.04
5	0	0.05	0.3	0.06

Although we secured excellent recoveries of calcium, it is not recommended that the method be applied to waters which would require less than 0.5 ml. of 0.01 N $KMnO_4$. We have tried the use of more dilute permanganate (0.005 and 0.001 N), but find that due to the dilution, considerable errors are introduced; excessive amounts of the permanganate are required to give the characteristic end point.

If the water contains less than 16 to 20 parts per million of Ca, 20 ml. should be evaporated to a volume of 10 ml., and the determination may be made upon this concentrate. If the calcium content is large, use a smaller sample and sufficient distilled water to bring the volume to 5 ml.

To calculate parts per million:

If sample = 5 ml. and $KMnO_4$ is 0.01 N
(ml. $KMnO_4$ – blank) × 0.2 × 200 = ppm. or
(ml. $KMnO_4$ – blank) × 40 = ppm.

If other volumes of sample are used, multiply the volume of $KMnO_4$ used (less blank) by the corresponding factor:

VOLUME OF SAMPLE, ML.	FACTOR
1	200
2	100
3	66.6
4	50

SUMMARY

The Clark-Collip modification of the Kramer-Tisdall method may be applied directly to waters of calcium content greater than 16 to 20 parts per million. Any technician doing blood chemistry should be already familiar with the method and should have all the necessary reagents and equipment. The method has given excellent results in this laboratory.

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STANDARDIZATION OF THE FOLIN-MALMROS MICRO BLOOD SUGAR METHOD FOR CAPILLARY AND VENOUS BLOOD AND ITS ADAPTION TO THE PHOTOELECTRIC PHOTOMETER*

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FREQUENT errors occur in visual colorimetry through variation in color sensitivity among observers and through fatigue in the observers. Several colorimetric methods have been introduced recently which utilize photoelectric cells to improve accuracy. In 1935 Goudsmit and Summerson[1] introduced a variable layer photoelectric comparison photometer based on the principle of the Duboscq colorimeter. Their method makes use of photoelectric cells for measuring the intensity of color in each cup independently. Differences in

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turbidity are also recorded as with any photometer. This flexible instrument with its improved accuracy should prove a distinct advantage in routine determination of blood sugars by such a method as the Folin-Malmros.

The Folin and Malmros[2] modification for determination of capillary blood sugar has advantages which makes it a valuable procedure. It is not only simple and accurate but has the additional advantage of slow fading of color. This slow fading permits a long series of determinations which can be read without appreciable loss. For these reasons, their method was peculiarly adaptable to the study we were making.

It became evident, however, in determining blood sugars in this way that certain factors must be investigated before it could be adapted to the photoelectric colorimeter. Two serious disadvantages were encountered among these factors, which had to be corrected. The first was the complicating one of a variable multicolored solution, i.e., the color of the Prussian blue and of the potassium ferricyanide. Folin and Malmros suggested the use of a yellow filter with the visual colorimeter to eliminate the color of the ferricyanide. A second disadvantage was a progressive loss from the calculated amount of sugar as the concentration of the sugar solution was increased. These two difficulties will be discussed later, as well as such other variables as time of heating, intensity of the light in the colorimeter, and the stability of the Prussian blue.

TABLE I

DUPLICATE DETERMINATIONS MADE ON CAPILLARY BLOODS DURING ROUTINE GLUCOSE TOLERANCE TEST AND ON KNOWN GLUCOSE SOLUTIONS

CAPILLARY BLOODS GLUCOSE TOLERANCE TESTS			KNOWN GLUCOSE SOLUTION	
Case S. K.	*Case L. J.*	*Case A. F.*	*7/19/37*	*7/20/37*
98.0	105	102	144	144
98.5		104		
144.9	134	159	185	185
147.0	137	160	215	215
143.9	200	141	264	264
143.9	200	143	299	295
178.6	179	111	345	345
178.6	178	113	375	378
137.9	135	116	409	400
140.8	136	117	418	418

TECHNIQUE

Capillary and oxalated venous blood were obtained in the usual way. The tehnique of the method used is essentially that recommended by Folin and Malmros, with the exception of the length of time for heating. Folin and Malmros gave directions for heating the tubes eight minutes. In this study the tubes were placed in the water-bath for twenty minutes. A 0.001 per cent glucose solution was used as a standard. The directions for use of the colorimeter given by Goudsmit and Summerson were followed. A yellow glass filter (supplied by Klett) proved to be of advantage. The deeply colored solutions in the Folin-Malmros method made a high intensity of light desirable, i.e., the A.C. transformer set at 6, and the galvanometer switch set at the No. 2 position. Although a

lower intensity of light gave essentially identical readings as the higher, the higher intensity increased the sensitivity of the colorimeter. It further resulted in a saving of time by making it possible to approach the null point of the galvanometer more rapidly. Two or more readings were made by racking the cup up or down. However, after some experience with the instrument, only one reading was necessary to insure accuracy. With proper care, 15 to 20 blood sugar determinations may be read without any loss of accuracy due to shifting of the instrument or to fading of the color. Scrupulous attention to details of operation and cleanliness of the instrument must be maintained.

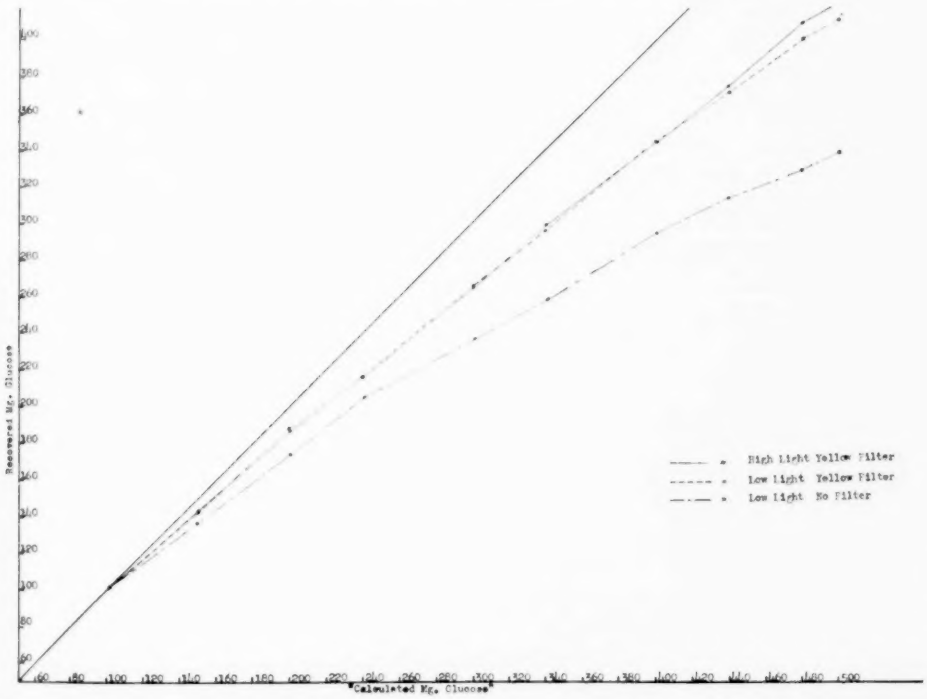

Fig. 1.—Recovery experiment on known glucose solutions showing effect of high light and low light intensity with a filter, and low light intensity without a filter.

DISCUSSION

A very satisfactory accuracy may be obtained with the use of the Folin-Malmros method when read with the photoelectric colorimeter. This is demonstrated in Table I, which shows a series of known glucose solutions determined on successive days. This same table shows a series of duplicate capillary blood sugars, taken during the course of routine dextrose tolerance tests. This degree of accuracy was difficult to approach with the visual colorimeter and especially so when the readings were made by different observers. Identical readings with the photoelectric colorimeter were readily obtained by different observers.

When variations occur in results obtained with the photoelectric colorimeter, they are significant of pipetting inaccuracies.

As indicated before, certain standards were selected somewhat arbitrarily because they offered advantages in constancy as well as speed of operation. With a high intensity of light no appreciable difference was noted whether the galvanometer switch was set at position 1 or 2. However, at position 1, somewhat lower readings were obtained when the intensity of the light was diminished. Such lower readings were especially characteristic with higher concentration of sugar. Fig. 1 demonstrates recoveries of a series of known solutions of glucose when readings are made with standard technique, with low light intensity, and without the yellow filter. It should be noted that the first two series of readings are almost identical except at higher concentrations when they tend to be a little lower with the low light. The percentage of difference, however, is minimal. Readings without the yellow filter are markedly lower than with the filter, and become progressively lower with the higher concentration of sugar. This difference is probably due to the progressively decreased yellow color of the ferricyanide solution as compared with the standard. A disturbance in the proportionality of the color of the solutions is reflected in the readings and produces a marked variation from the calculated readings. When the filter is used, this variation is largely obviated.

Studies made on the effect of the duration of heating indicate that eight to ten minutes is probably sufficient to complete the reduction of the ferricyanide to ferrocyanide. Twenty minutes' heating was used routinely, because, as suggested by Somogyi,[3] eight minutes may not give complete reduction. We found when blood filtrates were used the recoveries after eight minutes seemed to be lower and less constant than those obtained with twenty minutes' heating.

TABLE II

SHOWING THE STABILITY OF THE PROPORTIONALITY OF THE COLOR DEVELOPED IN THE FOLIN-MALMROS MODIFICATION CAPILLARY BLOOD SUGAR METHOD

	READING ON THE COLORIMETER SCALE		
NUMBER	IMMEDIATELY	HALF-HOUR	ONE HOUR
1	35.3	35.2	35.2
2	19.6	19.7	19.7
3	14.0	14.1	14.1
4	10.75	10.8	10.8
5	9.2	9.3	9.3
6	7.3	7.4	7.3
7	6.65	6.7	6.7
8	5.7	5.7	5.7
9	5.2	5.25	5.3
10	4.9	4.9	4.95
11	4.7	4.75	4.75

In Table II the stability of the proportionality of the Prussian blue is illustrated. Readings made immediately, in one-half hour and in one hour, show no evidence of fading and give essentially the same readings. This factor was significant when fifteen to twenty determinations were made in a given series.

Even after these variables were corrected and standardized, the readings obtained with the photoelectric cell colorimeter still did not approach the calculated readings and gave lower values than those obtained with the visual

colorimeter. The readings were consistent and produced a straight line curve when plotted on logarithmic paper. The depth of the color apparently did not follow Beer's law when applied to the response of the photoelectric cell. This discrepancy may be due to failure of quantitative production of the color or to interference by colloidal particles introduced with the iron-gum ghatti solution. In either case the factor was a constant for which a calibration curve (Fig. 2) was made. This curve was made for the 0.001 per cent standard. A 0.002 per

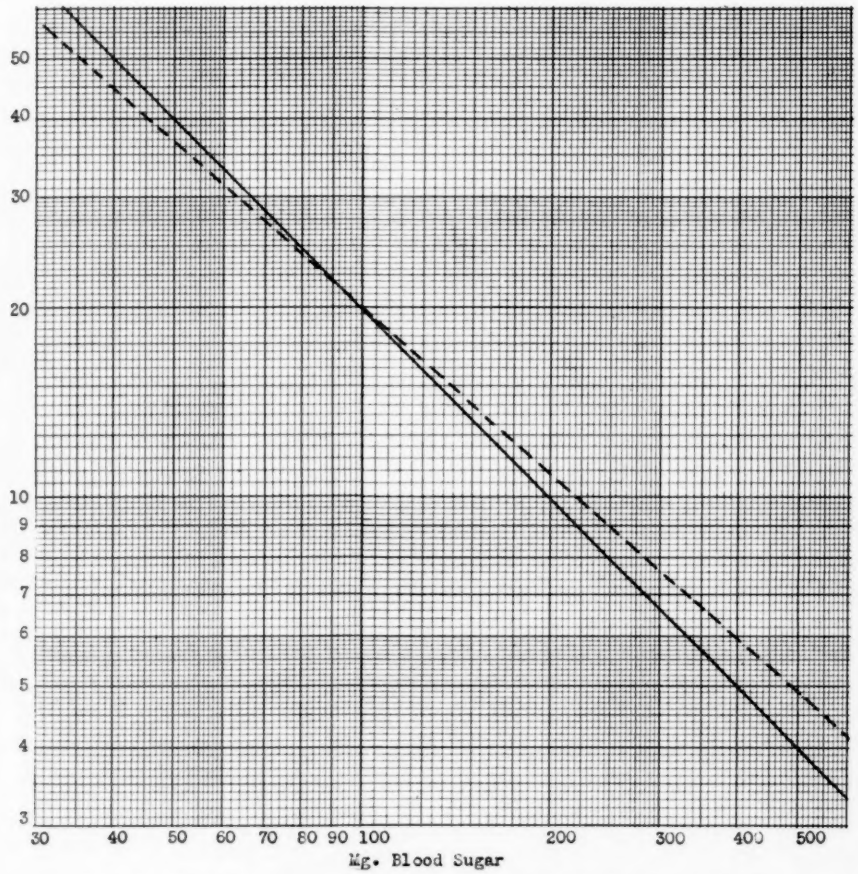

Fig. 2.—Calibration curve for 0.001 per cent standard glucose solution.

cent sugar standard may be used which will give accurate readings up to 250 mg. per cent of blood sugar without correction. This standard was discarded because it necessitated readjustment of the instrument with the change in standard. With the use of the calibration curve, accurate readings may be obtained as high as 500 mg. per cent, using only the 0.001 per cent standard. Fig. 2 shows the calibration curve. Table III shows readings on several blood sugar determinations corrected by using the calibration curve. When recoveries with the 0.001 per cent standard are corrected with the calibration curve, they will superimpose on recoveries with the double standard (0.002 per cent) up to a level of 250

TABLE III

SHOWING USE OF THE CALIBRATION CURVE IN HIGHER CONCENTRATIONS OF GLUCOSE

SPECIMEN	CALCULATED	RECOVERY	CORRECTED	DIFFERENCE
A. Mixture of 5 blood specimens		171	182	
B. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.33 per cent glucose)	258	232	261	plus 3
C. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.5 per cent glucose)	342	300	344	plus 2
D. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 0.67 per cent glucose)	425	361	425	minus 1
E. 0.1 c.c. of (1 c.c. of A plus 1 c.c. of 1 per cent glucose)	591	470	588	minus 3

mg. per cent. Above this level, correction must be made with the double standard also. We feel that the use of the single standard (0.001 per cent) is much simpler and gives as great a degree of accuracy as the use of several standards.

SUMMARY

The variable layer photoelectric colorimeter is adaptable to routine sugar determinations by the Folin-Malmros capillary method. The method is accurate and simple. A yellow filter is advantageous for securing consistent results. The readings with the colorimeter do not follow Beer's law, and a calibration curve is necessary for accurate results. A standardized technique permits a high degree of accuracy and makes feasible the use of the method for routine determinations of blood sugar.

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A METHOD FOR STAINING RICKETTSIA IN SECTIONS*

PROF. DR. SADAMI OCHI, SAPPORO, JAPAN

GIEMSA'S stain is generally used in the study of rickettsia in smear preparations. In well-made smears stained by the Giemsa method, one can demonstrate massively grouped rickettsia very well. In such smear preparations it is readily observed that the rickettsia are very definitely intracellular. Furthermore, Giemsa stain is used for the demonstration of rickettsia in tissues. Da Rocha-Lima has used Giemsa to stain *Rickettsia prowazeki* in sections from typhus: "the best stains are obtained in forty-eight hours, using 20 c.c. distilled water + 10 drops Giemsa solution, changing the staining solution every twenty-four hours. If one wishes rapid staining, 20 c.c. of water and 10 drops of Giemsa are used, changing the stain once or twice, for a period of two to three

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hours. The staining is made by flooding the slide, in a horizontal position." (Handb. d. path. Mikroorganismen **9**: 926, 1929.)

Notwithstanding that this method of staining with Giemsa has been proposed, it appears that staining of sections in the study of rickettsia is infrequently used. Possibly there is some valid ground for this. Nevertheless, if the manipulation of section staining is simple, the microbiologic study of stained sections might be important in rickettsia investigations, as in other, similar, cases.

In fact, well-stained sections could be very useful in microbiologic investigations. If, for example, some sort of colored bodies should be observed in stained sections, sufficient study could be made by serial sections to determine if they are, or are not, microbiologic in nature. It is self-evident that one cannot determine by stained smears whether the colored bodies in the tissue are a pathologic reaction product of the tissues or actual pathogenic microorganisms. But in stained sections one can observe simultaneously, and compare, the tissue changes and the stained bodies. Therefore, the examination of stained sections becomes more and more essential in such microbiologic studies, particularly if such bodies are readily stainable in sections.

The history with reference to the rickettsia is hereby omitted. Rickettsia have previously been found in cases of typhus. They have also been discovered, in Japan, in cases of tsutsugamushi disease. There are many reports of the detection of rickettsia in other diseases. H. Da Rocha-Lima says: "Some rickettsia, and among them *R. prowazeki*, *R. ruminantium*, *R.* (Dermacentroxenus) *rickettsia*, lead, in the host animal, an exclusively, or preponderingly, intracellular life and form juxtranuclear accumulations which remind one of the Chlamydozoa of Prowazek" (Handb. d. Path. Microorganismen **8**: 1347, 1930). In tsutsugamushi disease, the rickettsia found in smear preparations from experimental animals are chiefly in the endothelium and histiocytes. On the contrary, Prowazek's chlamydozoa, the so-called trachoma bodies, are found principally in the epithelial cells. Both, however, are very similar in morphology and in staining.

My method for staining trachoma bodies in sections is directly applicable to rickettsia in sections. My method was first published in 1910, in the Acta. Societ. Ophthal. Japon (**14**: April). By it, Gram-negative bacteria, as for example, the gonococcus, are readily stained in sections. Dr. Y. Onisi, in my clinic (Klin. Monatsbl. f. Augenh. **96**: June), used my method for the staining of trachoma bodies. Herewith is my method, which is applicable for staining rickettsia in sections:

1. The most suitable fixation solutions for pieces of tissue, for my staining method are:
   (a) Schaudinn's sublimate alcohol
   (b) Formalin solution

Commercial formalin	1
Distilled water	9

2. Celloidin imbedding was used in my earlier work, but paraffin is rather better: sections must not be more than 5 micra thick.

3. The staining solution consists of:

Kresylechtviolet (Grübler)	0.5
Glycerin	10.0
5 per cent carbolic acid	90.0
Distilled water	100.0

This staining solution is for sections, which are thoroughly fixed in sublimate alcohol. If, on the contrary, the sections are fixed in formalin solution, a concentrated staining solution is necessary.

Kresylechtviolet (Grübler)	0.5
Glycerin	10.0
5 per cent carbolic acid	90.0

The stain for these solutions is used as follows: the paraffin sections are laid, section side down, on the bottom of a flat dish. The ends of the slide are raised slightly, so that the sections do not quite touch the bottom of the dish. Then just sufficient staining solution is added to cover the section. This manipulation is designed to minimize precipitation of the stain on the section. The staining time is usually from ten to twenty minutes, at room temperature. More than twenty minutes does no harm. Then the sections are washed, first with distilled water, then differentiated with water acidulated with acetic acid. The acidulated water contains 5 drops acetic acid to 100 c.c. The excess of stain is thus removed from the section. For differentiation it usually suffices to leave the sections one minute in the acidulated water. Then the sections are again well washed to remove the acid. Then the water is absorbed from the stained sections with blotting paper, and they are air dried. Anilin-xylol (1:1) is poured on the sections to remove the last traces of water, and to clear them. Finally the anilin is removed with pure xylol and the sections are mounted in neutral balsam. The manipulations may be epitomized as follows:

1. Sections are imbedded in paraffin.
2. Kresylechtviolet solution, 10 to 20 minutes.
3. Wash with distilled water.
4. Treat with acidulated water.
5. Wash thoroughly with distilled water.
6. Dry sections.
7. Anilin-xylol.
8. Xylol.
9. Mount in neutral canada balsam.

By this procedure, rickettsia, in and out of the tissues, are detectable with strong magnification. The rickettsia appear mostly as tiny spherules, arranged occasionally as diplo- or streptococci. Also, one sees a few elongated rods, or dumbbell-shaped rickettsia. These elongated forms of rickettsia have deeply colored poles, with thinner central parts, but brightly colored.

Rickettsia appear to have intimate relation with the cells in the tissues; particularly one notices them in the substance of endothelial cells and histiocytes. In the parenchyma cells of experimental animals, as liver cells, of a

mouse, I have noticed rickettsia intracellularly. Obviously, they are also seen extracellularly, here and there.

The staining method, described above, is very simple, compared with the Giemsa method, and it can be employed for many purposes by anyone. By

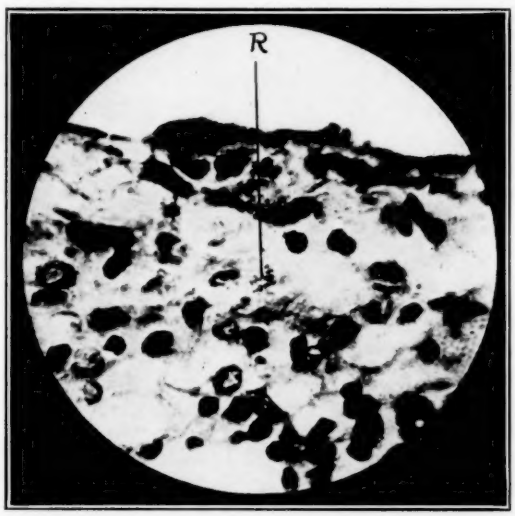

Fig. 1.—Peritoneum of mouse. In the center is a group of rickettsia (*R*). × 600.

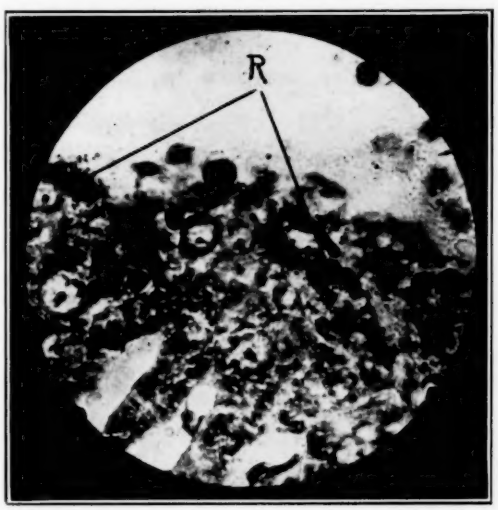

Fig. 2.—Superficial section of the liver of a mouse. Rickettsia (*R*), intra- and extracellular, are readily seen. × 600.

this procedure of staining sections, one may not only demonstrate rickettsia, but also one has the advantage of detecting, simultaneously, tissue changes as well. It is worthy of note that with my stain, the Kresylechtviolet has a metachromatic property. The mast cell granules are colored red brown. They are differentiated readily from rickettsia microscopically in that the latter are always stained a more or less dark violet. Also, in size, mast cell

granules and rickettsia are different. The former are somewhat larger than the granule-like rickettsia. I believe that on that account, my staining method for sections, with Kresylechtviolet is very simple, and will prove to be very useful for the study of rickettsia.

The figures show (Fig. 1) rickettsia in the peritoneum, and (Fig. 2) in the liver of a mouse, which were obtained from serial passage of tsutsugamushi disease, sent me by Prof. Dr. N. Ogata, Biological Institute of China Medical College.

A BODY TEMPERATURE REGULATING APPARATUS AND AN IMPROVED AXOGRAPH FOR ANIMAL EXPERIMENTS*

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TEMPERATURE REGULATOR

IN PERFORMING prolonged animal experiments under general anesthesia, the body temperature usually falls until such abnormal conditions prevail as to diminish the value of the results. To combat this, specially designed operating tables have been built with warming devices incorporated in them, or heating units have been put on the artificial respiration machines, when such are being used. None of these devices regulates the body temperature at any given temperature, but simply supplies extra heat. A more suitable method would be to fix the body temperature at the desired level by means of an automatically controlled device, in the same way as is done with water-baths, incubators, etc. I have, therefore, devised a simple electric thermostat, suitable for insertion into the rectum, which controls the heating unit in accordance with the changes in body temperature.

In Fig. 1 is shown a schematic drawing of the thermoregulator used. It is blown from Pyrex glass tubing of about 9 mm. external diameter, with walls of 1 mm. thickness. The general shape is that of an "L," with the bottom or horizontal portion bent slightly upward at an angle of about 85° from the vertical. The horizontal part is about 65 mm. long over all, and is closed off completely from the vertical portion, except for a small hole, 1.5 mm. in diameter, located at the lowermost point of lumen.

A much greater rise in the mercury than could be secured by its expansion alone is obtained by putting into the regulator a few drops of a liquid which boils at about the temperature sought. Its expansion in going from the liquid to gaseous state raises the mercury over a centimeter for each degree centigrade, in spite of the unusual width of the upright tube. Ethyl ether is a satisfactory liquid for temperatures around 37° C., but a somewhat more uniform rise at body temperatures is secured from a mixture of equal parts of the ether and carbon bisulfide.

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Filling and setting the instrument can be completed in very few minutes. The regulator is first filled almost to the top with mercury, then two or three drops of the expansion liquid are dropped on top of the mercury, the finger being placed over the end, taking care to trap a small bubble of air, and the whole is inverted. The expansion liquid and air rise, and by gentle tapping are made to pass through the small hole into the horizontal part. Then almost all of the mercury is poured out of the vertical portion, and it is restored to its upright position. The expansion liquid and air are thus trapped in the horizontal arm, and, as the liquid expands, it pushes the mercury up the ver-

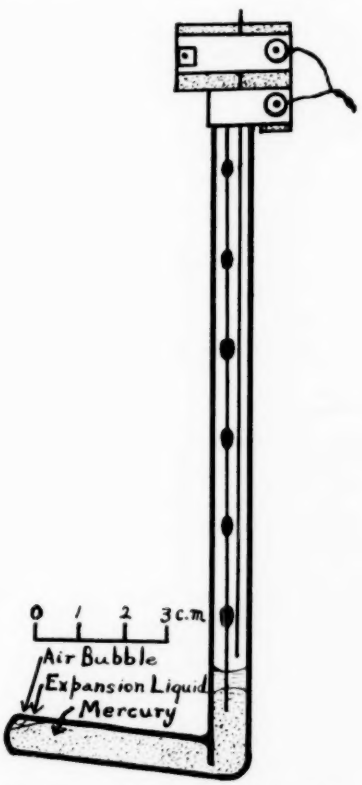

Fig. 1.—Diagrammatic cross-section of rectal thermostat.

tical arm. To set for temperature, the apparatus is immersed in a beaker of water about 1° C. hotter than the final temperature desired. The liquid expands as a gas, and, if too much has been put in, the mercury is completely blown out of the horizontal arm, allowing the excess gas to escape. The water is then allowed to cool to the desired temperature, during which process the mercury reenters the horizontal limb as the gas condenses. The adjustable contact rod is then pushed down to the surface of the mercury and clamped in that position. The contact rods are insulated from each other by glass beads threaded on the fixed rod. A drop of methyl salicylate put on top of the mercury prevents oxidation during operation of the thermostat.

In actual use, the horizontal limb is inserted into the rectum, and the thermostat held in place in the upright position by a clamp on a stand. The contacts of the thermostat control through a relay the heating pad or whatever source of external heat is being used. A very satisfactory and inexpensive relay for this purpose is the Precision Temperature Regulator,* which is so constructed that it requires only plugging in to the electric light socket to supply the current for both the heater and the thermostat. No secondary source of low voltage for the thermostat is required. As a heater, we ordinarily use any of the market brands of electrical heating pads, covered with rubber cloth to keep them dry, or the all-rubber one made by the Seamless Rubber Company. When working with small furry animals, such as cats, where heat transference from the pad to the animal is very slow, a pad of low heat capacity is best to prevent too much overswing in the regulation of the temperature. Clipping the hair off the back also increases the speed of heat transfer and makes the regulation more sensitive. Although this apparatus has been used only in animal experiments, there can be little doubt that the principles involved would be equally applicable to the maintenance of body temperature of patients under conditions of shock, collapse, prolonged poisoning, etc., where this is a major factor in promoting recovery of the patient, and ordinarily requires continuous nursing attention.

AN IMPROVED AXOGRAPH

In making kymographic records, particularly of blood pressure, where the amount of rise or fall is important, it is a great convenience to have automatically drawn on the record a scale from which the magnitude of the changes can be read off at a glance. An instrument for this purpose was described and named an axograph by Gautrelet[1] some years ago. Gautrelet's apparatus had the disadvantage that it was mounted rigidly on a clamp and spring bar, so that any irregularities in the paper, or lack of exact parallelism in the support rod, prevented contact over the whole width of the paper. It did not prove satisfactory for general use. The axograph pictured in Fig. 2 is simpler to make and free from this disadvantage.

As shown in the schematic cross-section, the supporting rod fits into the sleeve of the axograph. A round pin passes through this sleeve, engaging in a slot in the support rod, thus fastening it in, but permitting complete revolution of the axograph on the rod. In addition, the supporting rod is placed nearer to one end of the axograph, so that when the rod is held horizontally the face of the axograph hangs in the vertical position by the force of gravity and parallels the surface of the paper. The revolving bar of the axograph is very simply constructed by using a gear bar of the desired length, and about 15 mm. in diameter, and cutting it out on a lathe to leave circles of teeth 5 mm. apart along its length. The appearance of this in cross-section at the level of one row of the teeth is shown in Fig. 2. When the axograph is held in contact with the smoked paper, the bar revolves, drawing a series of dotted lines 5 mm. apart on the paper, which correspond to 10 mm. of blood

*This regulator and the thermostat can be obtained from the Eastern Engineering Company, New Haven, Conn.

pressure when the ordinary type mercury manometer is used. In having the bottom line of dots at the zero level, the blood pressure can be read off very readily, as shown on the record in Fig. 2. The revolving bar can be made whatever length is required for the width of the kymograph paper customarily used, the one pictured being used for 10-inch paper. The advantage of this axograph over Gautrelet's is in the swivel joint in the supporting rod, which permits the revolving bar to swing out of its vertical plane and remain in contact with the entire width of the paper, in spite of irregularities on the surface, or wrinkles that are so frequent on long paper kymographs.

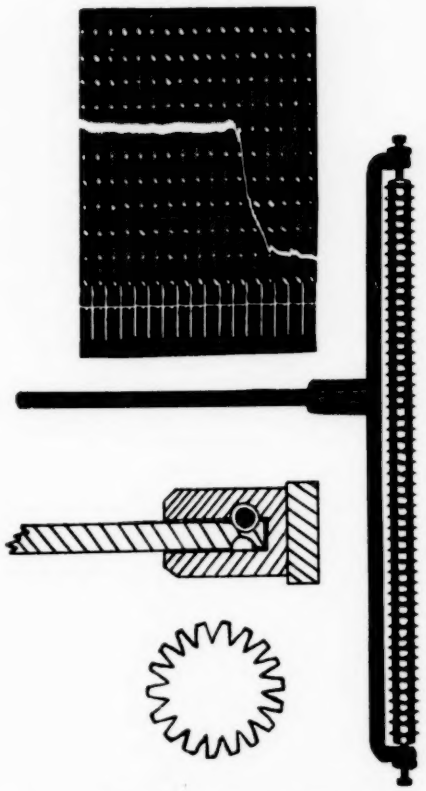

Fig. 2.—Photograph of axograph, cross-section drawings of revolving bar and central pivot construction (not to scale), and typical blood pressure tracing, showing the dotted lines drawn on the record by the axograph.

In actual use, the axograph is mounted on a long paper kymograph, clamped to an upright rod at the far side of the apparatus. It is swung into contact with the paper between the drums, with sufficient pressure to give firm contact. The drum is then quickly revolved through a complete turn of the paper, dotting in the axograph record as it revolves. Then the instrument may be swung away from the kymograph so as to be out of the way when the paper is taken off or fresh paper put on.

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A SIMPLIFIED METHOD OF CORRECTING THE SEDIMENTATION RATE FOR THE EFFECT OF CELL VOLUME*

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THE desirability or otherwise of correcting the sedimentation rate for the effect of cell volume was investigated at this sanatorium, and a simplified method of carrying out this correction was devised, which is briefly outlined herewith. In this method the one-hour sedimentation reading and the cell volume are determined on the same blood sample and in the same tube. As indicated elsewhere[1] this method gives essentially the same results as the Gram[2] and Rourke-Ernstene[4] techniques for obtaining the corrected sedimentation rate, although the numerical scales used in the three methods differ.

In preliminary experiments during the summer of 1933 undiluted oxalated blood was employed, and curves were prepared to correct for the effect of cell volume upon the one-hour sedimentation readings. For two reasons this method was not used in clinical practice: (a) Using dry oxalate as anticoagulant, occasional samples contained specks of clumped red cells, and results from such samples may not be reliable. This difficulty was not encountered when isotonic oxalate or citrate solution was employed. (b) It was found by trial that for the more rapidly settling bloods the correction curves became erratic when the cell volume exceeded 45 per cent; Rourke and Ernstene[4] encountered the same effect in undiluted heparinized blood.†

In the technique described below isotonic citrate solution is used as anticoagulant, the blood samples used in the test containing 15 per cent by volume of citrate solution. Thus a 45 per cent cell volume in the citrated sample corresponds to a cell volume of 53 per cent in the undiluted blood, and higher cell volumes than this are rare. By this means consistent correction curves were obtained over the range of cell volume normally encountered, for both slowly and rapidly settling bloods.

The correction chart to be used in this method is based on data obtained from blood samples from 30 patients. In each case 15 c.c. of blood was added to 1.8 c.c. of 3.8 per cent citrate; a portion was centrifuged to determine the cell volume and from this the citrate concentration in the plasma was calculated. The bulk of the sample was then divided into five portions; by removal or addition of calculated amounts of plasma, and addition of calculated amounts

*From the Mara Laboratories at the Queen Alexandra Sanatorium, London, Ontario.
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†This difficulty was overlooked by Wintrobe and Landsberg[5] who employed undiluted oxalated blood in their C.S.R. method, since in preparing their correction curves they used only blood from normal persons. Also these authors assume that in the 100 mm. tubes employed the correction curves for rapidly settling bloods have the same general form as those for slowly settling bloods; this is not in accord with the experimental evidence obtained in this work.

of citrate solution, the cell volumes in the five portions were adjusted to 25, 29, 35, 40, and 45 per cent, and the citrate concentration in the plasma of each sample was brought to the appropriate amount. Adjustment of the citrate concentration in the plasma is necessary, since when in routine work blood is drawn from patients, the citrate concentration in the plasma is influenced by the per cent cell volume of the blood. The well mixed samples were placed in flat bottomed sedimentation tubes of 4 mm. bore, which gave a blood column 100 mm. high. After one hour the observed sedimentation was recorded in

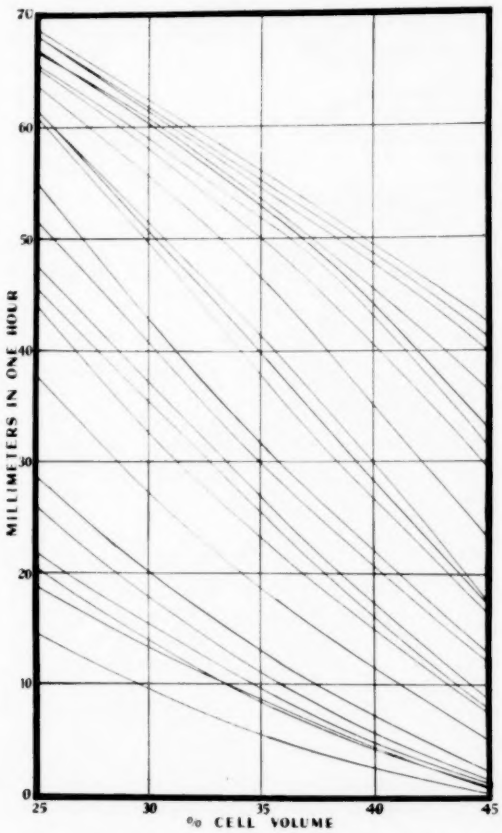

Fig. 1.—Influence of in vitro change in cell volume upon the sedimentation rate.

millimeters, the samples were then centrifuged thirty minutes at 3,000 r.p.m., and the percentage volume of packed red cells read off directly. The results thus obtained are given in Fig. 1; three blood samples gave curves so closely alike that they are represented by only one line in the figure; the same is true for two other pairs of curves. No attempt was made to specially select the patients, since it is clear from the results of Gram[3] and Rourke and Ernstene[4] that the in vitro influence of cell volume upon the sedimentation rate is a consistent one, which is not related to the particular infection. By averaging the results of Fig. 1, the final correction chart given in Fig. 2 is obtained.

DETAILS OF TECHNIQUE

In routine use of this method, 2 c.c. of blood is added to 0.35 c.c. of 3.8 per cent citrate. Accurate measurement is essential, especially of the citrate, since the same sample is used both for a cell volume estimation and for the sedimentation test. Using a 2 c.c. long precision type of syringe and the technique indicated, we find that the syringe transfers 0.05 ± 0.01 c.c. of citrate to the blood sample;* the remainder of the citrate, 0.30 c.c., is placed in the small tube which receives the blood. The sedimentation tubes are 11 c.c. long and of 4 mm. bore; they may be graduated in millimeters to give a blood column 100 mm. high—the Wintrobe hematocrit tube[6] is satisfactory, or the tubes may have a single graduation mark 100 mm. above the floor of the tube, readings being taken by a millimeter scale held against the tube.

METHOD

Use a 2 c.c. precision type syringe, dry and sterile, and a 1 inch 20-gauge needle. Draw sterile 3.8 per cent citrate into the syringe, and run the piston up and down, so as to wet all the inside of the syringe. Then drive the piston completely in while the needle is pointed upward, so that all air bubbles are removed and the needle is left full of citrate.

Enter a vein and draw blood accurately to the 2 c.c. mark. Withdraw from vein, quickly detach and discard the needle, invert the syringe over a small tube containing the additional volume (0.30 c.c.) of 3.8 per cent sodium citrate, and press in the piston so that the blood flows vertically downward into the citrate in the tube. Do not expel the blood against the side of the tube. Immediately close the tube with a clean cork and invert and rotate it 10 times. The tube containing the citrate must be uncorked and conveniently placed before commencing to draw the blood, as there should be no delay in the operation. The sample should not be used for fifteen minutes, so that it may reach room temperature. Within five hours from the time of drawing the blood, it is well mixed by gently inverting and rotating the tube for at least ten times; it is well to hold the tube by the corked end, to avoid warming the sample by the heat of the hand; with a capillary pipette fill the sedimentation tube to the zero mark, taking care to avoid air bubbles. After standing one hour in a perfectly vertical position, the degree of sedimentation is read off in millimeters. The tube is then centrifuged 30 minutes at 3,000 r.p.m., and the percentage volume of packed red cells is read off directly from a millimeter scale.

From the graph of correction curves the observed sedimentation rate is corrected to standard cell volume, i.e., to the 37 per cent cell volume line of Fig. 2. This cell volume, 37 per cent, is chosen as it corresponds to 42.5 per cent cell volume in the undiluted blood, which, using Gram's figures, would be the mean of the average cell volumes for both men and women. To use the graph in routine work that point upon the graph is located which corresponds to both the cell

*The amount of citrate transferred to the blood is found by blank trials with this technique, using N/10 NaOH in place of citrate, and distilled water in place of blood. The volume of NaOH carried over into the water is found by titration with acid.

volume and the observed sedimentation rate of the blood sample; the curve upon which this point lies, or the interpolated curve if it lies between two curves, is then followed to the standard cell volume (37 per cent) line, where the position of the curve gives the sedimentation rate for standard cell volume. For example, if the observed sedimentation is 20 mm. and the cell volume 33 per cent, find the figure 20 on the left hand scale of Fig. 2, and follow the horizontal line opposite this figure until vertically over the figure 33 of the bottom line. The point thus located is found to lie on one of the curves; following this curve down to the

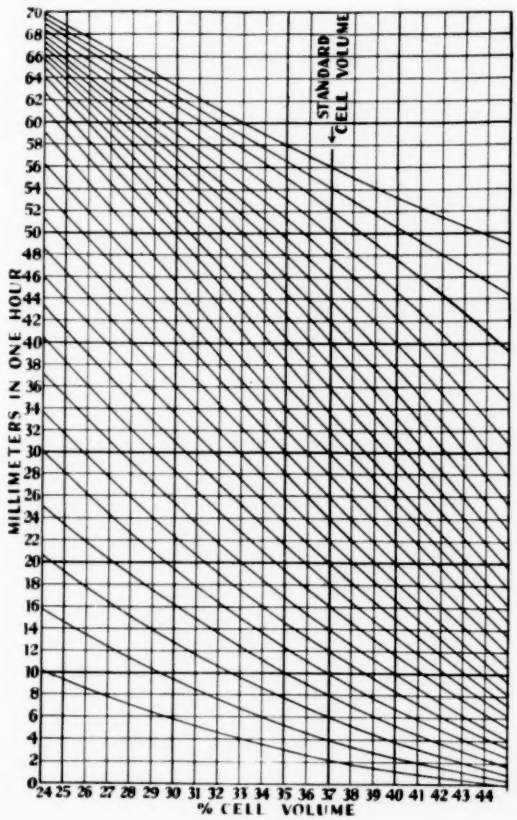

Fig. 2.—Chart for correcting observed sedimentation rates to standard cell volume (37 per cent). The cell volume figures refer to the citrated blood, 85 vol. blood to 15 vol. of 3.8 per cent citrate, used in the test.

standard cell volume line, the two intersect at a point opposite figure 14 of the left hand scale. This figure gives the sedimentation rate, corrected to standard cell volume.

NORMAL STANDARDS

For corrected sedimentation rates obtained by the method described, the following may be taken as the standards for both men and women.

Normal	1 to 7 mm.
Borderline	8 to 10 mm.
Pathologic	11 mm. and over.

Correction for cell volume has been advocated by several authors[3, 4] in the belief that it eliminates inaccuracies resulting from variations in the red cell content of the blood. However, it should be kept in mind that all methods of correcting for cell volume are based on in vitro findings, when the ratio of red cells to plasma is artificially altered. In practice, there may be other factors involved,[2] so that correction for cell volume may introduce greater errors than it eliminates. It was to investigate this point that the foregoing method was devised; comparisons of clinical and x-ray data to the sedimentation rates were made by Dr. D. W. Crombie. The results obtained indicate that correction for cell volume is in general of advantage for blood containing over 45 per cent cell volume, i.e., 39.2 per cent cell volume in the citrated sample, especially when the sedimentation rate is relatively low. On the other hand, correction for cell volume is in general uncalled for or undesirable in the case of blood having normal or subnormal cell volume.

Acknowledgment.—The authors are indebted to Dr. D. W. Crombie, Medical Superintendent, and to Dr. P. M. Andrus, Director of the Mara Laboratories at the Queen Alexandra Sanatorium, who suggested and made possible this work. The technical assistance of Mr. R. Comrie is also acknowledged.

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A DIRECT READING NUL POINT AMPLIFIER FOR MEASURING HYDROGEN ION CONCENTRATIONS AND OXIDATION-REDUCTION POTENTIALS*

F. Lyle Wynd, Ph.D., and Philip L. Varney, Ph.D., St. Louis, Mo.

SKOW and Wynd[1] have published a description of a single tube amplifier for use in the determination of glass electrode potentials, which is characterized by operating at a low filament temperature, low plate voltage, and with a grid bias equal to the free grid potential. The present paper describes modifications of the apparatus whereby the measuring part of the circuit can be so adjusted as to cause the apparent potential value to equal the pH of the unknown solution, irrespective of the temperature.

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Fig. 1 shows that the amplifying part of the circuit is similar to that previously described. A potentiometer (R8, R9, B5) has been inserted in the measuring part of the circuit to cancel the calibration constant of the system.

The battery (BA) of the measuring potentiometer applies its potential to the calibrated resistance through appropriate resistances (R10, R11), so that the voltage drop between successive contact terminals of the potentiometer is no longer 0.100 volt, as usual, but is equal to the theoretical change in millivolts per pH unit. The authors have found it convenient to distribute R11 between fixed and variable resistances so as to allow R10 to carry only that part of the load needed to correct the total resistance for the effects of temperature in the pH :millivolt ratio.

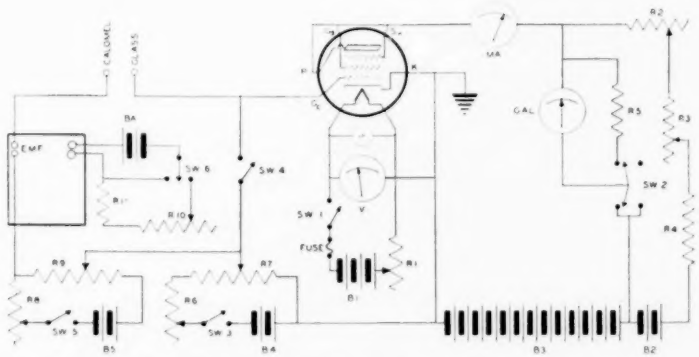

Fig. 1.—The theoretical wiring diagram of the amplifier. Type -89 vacuum tube; 0-1 D. C. milliammeter; 0-6 D. C. voltmeter, 1000 ohms per volt; Gal., Leeds and Northrup 2320-d galvanometer; R1, 20 ohms; R2, 400 ohms; R3, 10,000 ohms; R4, 1,500 ohms; R5, 100 ohms; R6, 400 ohms; R7, 15,000 ohms; R8, 2,000 ohms; R9, 20,000 ohms; Sw. 1, 2, 6, and reversing, 4 p.d.t. Federal anticapacity switches; Sw. 4, s.p.d.t. Federal anticapacity switch; B1, 6-volt storage battery; B2, B4, B5, 3 to 4.5 volts B batteries; B3, 19.5 volts from radio B batteries; BA, potentiometer battery.

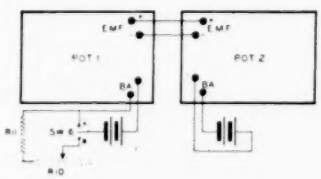

Fig. 2.—Method of calibrating the temperature correction dial.

The calibration of R10 is accomplished empirically by balancing two potentiometers against each other as shown in Fig. 2. With Switch 6 at position *A*, Potentiometer 1 is standardized as usual by its standard cell. Potentiometer 2 is then standardized with the same standard cell. Switch 6 is then thrown to position *B*. Potentiometer 1 is then set to read 1.000 volt, and Potentiometer 2 is set to read 0.5733 volt. Adjust R10 until balance is obtained. The range of Potentiometer 1 now varies numerically parallel with pH at a temperature of 16° C. An appropriate mark is made on the instrument panel to record this position. Potentiometer 2 is then set to read 0.5773 (voltage change for each ten pH units at a temperature of 18° C.) and the process described above re-

peated. The necessary values for temperature corrections from 16° C. to 42° C. may be obtained from Table I.

TABLE I

THE CHANGES IN VOLTAGE (D) PER pH UNIT AT DIFFERENT TEMPERATURES

TEMP. ° C.	VOLTAGE CHANGES PER pH UNIT	TEMP. ° C.	VOLTAGE CHANGES PER pH UNIT
12	0.0565	28	0.0597
14	0.0569	30	0.0601
16	0.0573	32	0.0605
18	0.0577	34	0.0609
20	0.0581	36	0.0613
22	0.0585	38	0.0617
24	0.0589	40	0.0621
26	0.0593	42	0.0625

The resistance values of R10 and R11 will depend on the type of measuring potentiometer (Potentiometer 1) used. For this reason a preliminary determination of the total ohms resistance needed should be made, using a resistance box in place of R10 and R11. Providing an instrument such as Leeds and Northrup Potentiometer 7655 is used, R10 should be approximately 125 ohms, while R11 can be conveniently distributed between a 350 ohm fixed resistance and a 0 to 100 ohm variable resistance. For a Type K potentiometer these values should be 30, 50, and 0 to 50 respectively.

It is obvious that the measuring potentiometer must be standardized with Switch 6 at position *A*, and that in this position of the switch the E. M. F. readings on unknown solutions are expressed in millivolts. This switch enables the operator to instantly convert the apparatus from a direct reading pH meter to a standard potentiometer for the determination of pH by calculations or of oxidation-reduction potentials.

Assuming that the apparatus is wired according to Fig. 3, the operation is as follows:

1. Depress Switch 1. This closes the circuits indicated in Fig. 1 by Switches 1 and 3. When tube 89 is used, adjust R1 until the milliammeter indicates a plate current of 0.6 to 0.8 milliampere. If tube 38 is used, adjust until the plate current is 0.3 to 0.4. When R1 is set, it needs no further adjustment for many months.

2. When the plate current has become constant (three to five minutes), depress Switch 2. This completes the galvanometer circuit so that the major portion of the current is shunted past the galvanometer, thus protecting this instrument until the circuit is balanced. Balance with R2 and R3 until the galvanometer reads zero, or approximately zero. Slight instability of the galvanometer may be observed at this stage, but this in no way affects the final accuracy of the readings. The balancing of the galvanometer in this condition of low sensitivity is necessary only during the process of determining the original zero point. It is not necessary during the determination of successive pH readings.

3. Depress Switch 4 and balance by adjusting R6 and R7 until the galvanometer needle is stabilized. This can be at any point on the galvanometer dial, but for convenience should be at the zero position. Open Switch 4.

4. Raise Switch 2 to the upper position (position of maximum sensistivity of the galvanometer) and rebalance as in Step 3. Switch 2 remains in the position of maximum sensitivity during the determination of pH readings.

5. Raise Switch 6 to the upper position, and standardize the measuring potentiometer. If readings are to be made in terms of volts, Switch 6 remains in this position. The unknown solution, in which the glass or platinum electrode is inserted, is now bridged to the calomel cell. For this purpose a permanent bridge, filled with saturated potassium chloride solution, consisting of a pyrex "U" tube in the ends of which have been sealed porous porcelain plugs, can

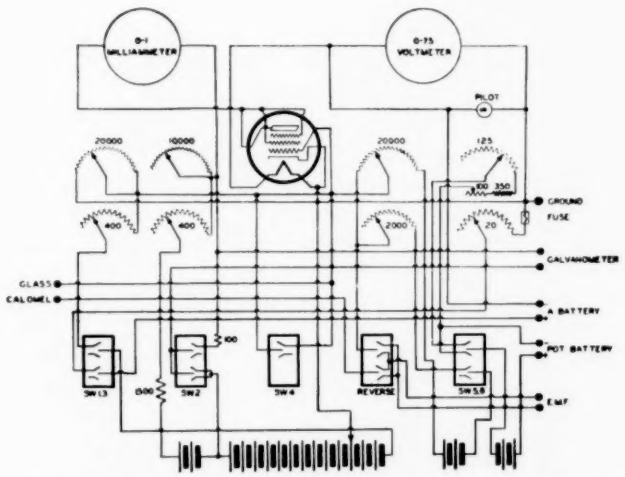

Fig. 3.—Actual hook-up of the amplifier. The terminals indicated by "glass" and "calomel" are attached to the jack connections of the electrode set-up illustrated in Fig. 6. The terminals indicated as "ground," "galvanometer," "battery," "pot. battery," and "E.M.F." are led through the metal cabinet by porcelain insulators 2 inches long as indicated in Fig. 4. The parts of the circuit thus exposed are sufficiently insensitive to shielding to enable the indicated connections to be made externally. The "ground" terminal is soldered to a water pipe. The "galvanometer" terminals are attached to the indicated terminals of the galvanometer as indicated in Fig. 5. The "A battery" terminals are attached to a 6-volt storage battery. The "pot. battery" terminals are attached to the battery terminals of the measuring potentiometer, the battery itself is within the cabinet. The "E.M.F." terminals are attached to the corresponding terminals of the measuring potentiometer, as indicated in Fig. 5.

conveniently be used. The measuring potentiometer is adjusted until the galvanometer needle shows no deflection upon depressing Switch 4. This again can be at any point on the galvanometer scale.

6. If readings are desired directly in pH units, first standardize the measuring potentiometer as in Step 5, with Switch 6 in the upper position. Then depress Switch 6 and bridge a solution of known pH (as, for instance, M/20 potassium phthalate buffer—pH 3.97) to the calomel cell. Set the temperature correction dial R10 to the position corresponding to the temperature of the known buffer, and balance with R8 and R9 until the galvanometer needle shows no deflection on closing Switch 4. Unknown solutions may now be sub-

stituted for the known buffer and their hydrogen ion concentration read directly. For the greatest accuracy, the pH of the known standardizing buffer should approximate that of the unknown solution. If well insulated electrodes are used and the tube is thoroughly warmed, readings accurate to ±0.003 pH unit can be made. In the determination of successive pH readings, it is only necessary to rinse the glass electrodes, substitute another solution, and balance with the measuring potentiometer until the galvanometer needle shows no deflection when Switch 4 is tapped downward. Hence, successive readings can be made in a few seconds.

When the pH is being determined by calculation from the readings in millivolts, the readings of a standard buffer must first be obtained. The following formulas are then used. Switch R, in Fig. 4, reverses the terminals of the measuring potentiometer.

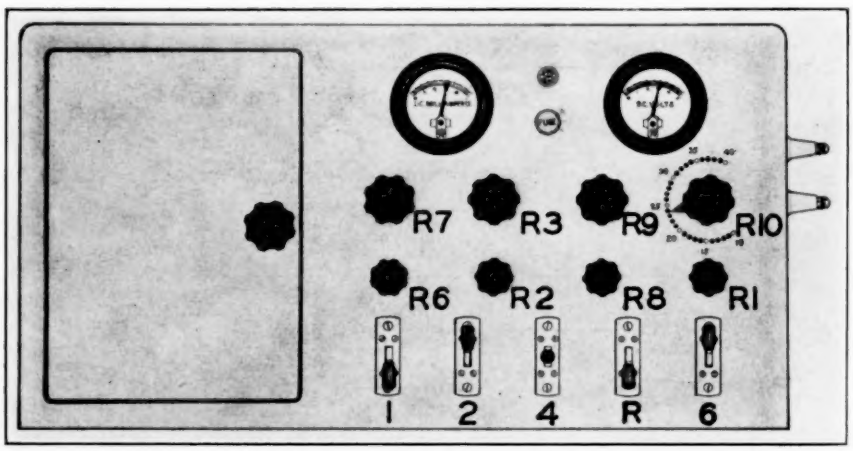

Fig. 4.—Diagram of front of box showing position of the switches and dials. These positions correspond to those shown in Fig. 3.

1. If the observed potential is greater than that of the standard buffer (higher pH, the reversing Switch R will be down).

$$\text{Unknown pH} = C + \frac{(A - B)}{D}$$

2. If the observed potential is less than that of the standard buffer (lower pH, but Switch R down),

$$\text{Unknown pH} = C - \frac{(B - A)}{D}$$

3. When reversing Switch R is up (very low pH values):

$$\text{Unknown pH} = C - \frac{(A + B)}{D}$$

where A = potential of the unknown; B = potential of the standard buffer; C = pH of the standard buffer, and D = voltage change per pH unit at the temperature of the unknown solution (see Table I).

Switch 6 in Fig. 4 is so wired that when it is in the upper position, the circuit indicated by Switch 5 in Fig. 1 is broken. This causes the potentiometer (R9, B5) to function only when pH readings are being made directly.

Since a galvanometer is needed for the preliminary standardization of the measuring potentiometer, it follows that two galvanometers are needed for the complete apparatus, provided a potentiometer with a built-in galvanometer is used. A simple modification of the wiring of the potentiometer will permit the built-in galvanometer to be used first to standardize the measuring potentiometer and then by throwing a switch to function in the plate circuit of the amplifier. Fig. 5 shows the changes necessary for a Leeds and Northrup potentiometer Type 7655.

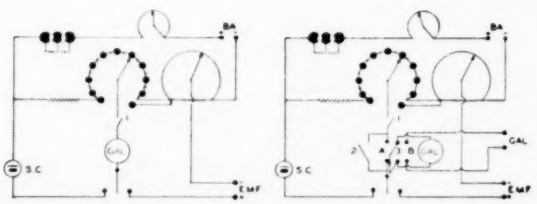

Fig. 5.—Wiring changes of built-in-galvanometer type potentiometer to obviate the use of a second galvanometer. The diagram to the left indicates the original potentiometer wiring, the figure on the right indicates the modifications. The switch indicated may be a double pole, double throw Federal anticapacity switch mounted at any convenient place in the potentiometer box.

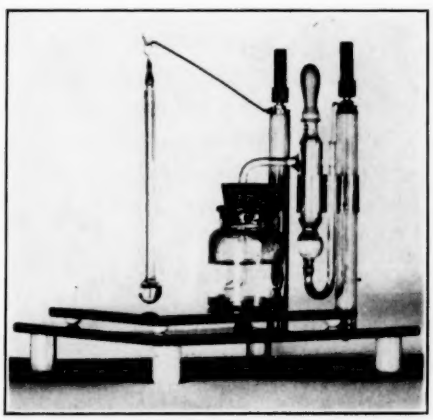

Fig. 6.—Electrode set-up. The jack connections at the ends of the vertical insulators are attached to the corresponding terminals indicated in Fig. 3. The electrode set-up is, of course, shielded within the grounded metal cabinet.

The electrode set-up is shown in Fig. 6. By means of the double shank glass electrode,[2] errors due to electrical leakage across the electrode surfaces are reduced to an insignificant magnitude. The side arm of the calomel reference electrode dips into a saturated potassium chloride solution, contained in a bottle fitted with a two-hole rubber stopper. The upper portion of the bottle is smeared with lanolin to prevent creeping of the potassium chloride. The glass areas of the permanent bridge should likewise be covered with a very thin layer of lanolin, so that the porcelain tips can be kept in saturated potassium chloride solution when the apparatus is not in use.

The very great resistances of the type of glass electrodes used necessitate careful shielding and grounding of the apparatus. The metal cabinet shown in Fig. 4, connected to the circuit as shown in Fig. 3, is grounded.

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A METHOD FOR UTILIZING ILLUMINATING GAS IN THE BROWN, FILDES AND MC INTOSH OR OTHER ANAEROBE JARS OF THE LAIDLAW PRINCIPLE*

John H. Brewer, M.A., and J. Howard Brown, Ph.D., Baltimore, Md.

THE most satisfactory method for the cultivation of anaerobes makes use of some type of jar in which the oxygen is completely burned out by means of a catalyst, originally employed by Laidlaw (1915). This method eliminates the trace of oxygen, which is always present in evacuation and replacement systems, and which seriously interferes with the cultivation of the most strict anaerobes, such as *Cl. novyi*. The principal objections to the use of these jars have been the initial cost of equipment and the earlier explosions which occurred with improperly designed and poorly constructed jars. The danger has been eliminated in more recently designed equipment.

Hall (1929) reviews the success and failure of several workers in using illuminating gas for replacement after evacuation, although he makes no mention of the use of this gas in catalyzer systems. We have tried over a period of three years to use gas from the laboratory jet in the Fildes and McIntosh (1921) and Brown (1921) jars, but without complete success until this past year. It was supposed that the gas had some toxic effect as has been reported by Kladakis (1890), Ludwig (1918), and Frankland (1889). Kladakis is the only one of these who used an anaerobic organism. His technique of bubbling the gas through the small amount of medium for thirty minutes would saturate it with the carbon dioxide present, so that the pH would not be in the proper range for growth to occur. Ludwig and Frankland used aerobic or facultative organisms whose growth would naturally be inhibited to some degree by anaerobic conditions. Their results were largely due to a lack of oxygen, and not to toxic action of the gas.

Wurtz and Foureur (1889) used illuminating gas for freeing the medium of air, and for keeping the air out of the test tube until they made an inoculation under an oil seal; they did not actually cultivate the organism in the presence of the gas.

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Bulloch (1900) and Wilson (1917) employed illuminating gas to flush the air out of special containers; then used pyrogallic acid and alkali to remove the remaining oxygen. The large amount of alkali used (109 gm. to 145 c.c. of water) was sufficient to absorb much of the gas so that the pH would not be lowered. Wilson reported excellent growth of *Cl. sporogenes, Cl. septique, Cl. histolyticum, Cl. oedematiens,* and *Cl. tetani.* In his system there was unquestionably some gas present, but the toxic effect of the carbon dioxide was certainly eliminated by the alkali present.

Holker's (1920) success in using coal gas is readily explained in the earlier part of his paper: "At the outset, it is to be pointed out that the bacteria are not grown in an atmosphere of coal gas, nor were they grown in an atmosphere of hydrogen. . . . The coal gas or the hydrogen, as the case may be, serves merely to wash the oxygen from the vessel. When the vessel is finally closed off in vacuum, both the oxygen and coal gas remaining are very small in amount."

Brown, in describing the use of his jar, suggests that the hydrogen be allowed to diffuse into the jar at about five pounds' pressure. The gas pressure at a laboratory jet is usually about one-fortieth this pressure. Because of this low pressure, not enough of the gas will flow into the jar to start combustion. If the jar is attached to an ordinary inexpensive water vacuum pump for about one minute and then attached to the gas jet, enough gas will be drawn in to start combustion. A manometer need not be used, but if convenient may be employed and the jar evacuated to about 50 cm. of mercury. This is not to be confused with an evacuation replacement method; it is only to get sufficient gas into the jar, under low pressure, to initiate combustion. The jar is allowed to remain attached to the jet after the electric current is turned on for about thirty to forty-five minutes, then the electric and gas currents turned off and the jar sealed.

Since the gases found in municipal supplies are usually ethane, methane, hydrogen, carbon monoxide, and illuminants, all of which burn to carbon dioxide and water, there will be formed an excess of carbon dioxide under a slight pressure which will lower the pH of the media about 0.4. Valley and Rettger (1927) made a very exhaustive study of the effect of carbon dioxide on bacteria. This study includes *Cl. sporogenes, Cl. welchii, Cl. tetani,* and *Cl. putrificum,* and

TABLE I

TYPES OF GAS SUITABLE FOR USE AS DESCRIBED

	NEW YORK	PHILADELPHIA	BALTIMORE	CINCINNATI*		CHICAGO	ST. LOUIS	LOS ANGELES
Illuminants	3.7†	5.9	3.9	0.1	1.5	2.1	4.0	
Hydrogen	45.4	37.7	36.1	9.8	18.7	24.6	25.6	
Methane		17.6	24.6	66.0	67.5	55.7	47.5	80.0
Ethane	26.1	1.9		8.0	4.7	6.9	9.2	16.6
Carbon dioxide	2.4	5.2	3.5	2.9	1.4	1.0	1.0	1.0
Carbon monoxide	8.2	16.0	13.0	8.4	3.1	2.8	2.9	
Oxygen	0.9	0.9	1.0	0.2	0.4	0.3	0.7	0.7
Nitrogen	13.3	14.8	17.9	4.6	2.7	6.7	9.1	1.7

*Two types of gas are used in Cincinnati.
†Analyses are in per cent by volume.

their conclusions are that any harmful action brought about on bacteria by carbon dioxide is due to increased hydrogen ion concentration, although they found that a certain amount of carbon dioxide actually favored the growth of bacteria. To offset this increase in hydrogen ions the media may be buffered,

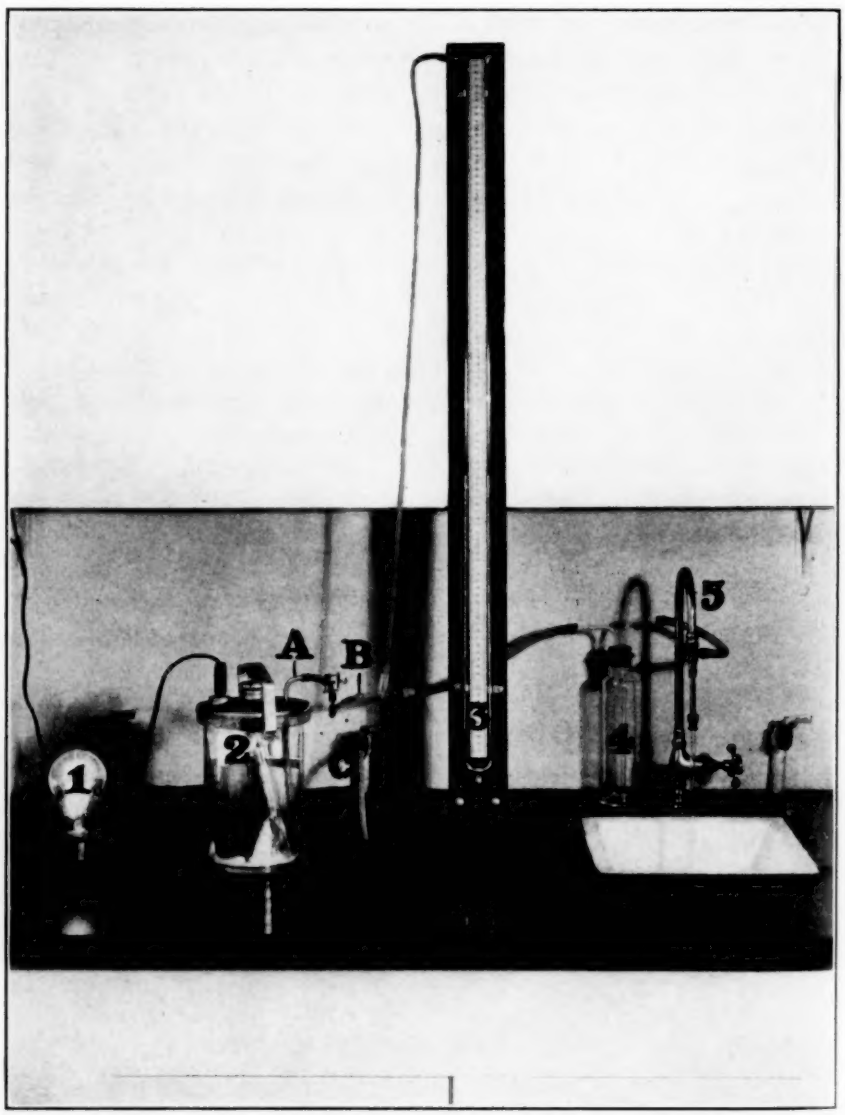

Fig. 1.—*1*, A 32-candle power carbon filament lamp in series as a resistance unit; *2*, improved type Brown jar, manufactured by the Scientific Equipment Co., Philadelphia; *3*, manometer connected in the vacuum line by T-tube; *4*, water trap; *5*, water vacuum pump. *A*, Outlet to jar. Rubber tube leads to bottom of jar to aid diffusion; *B*, vacuum connection; *C*, illuminating gas jet connection (Photograph Courtesy of Hynson, Westcott & Dunning, Baltimore).

but we have found it more convenient to enclose a test tube of soda lime which may be used repeatedly for at least five weeks. The use of soda lime is perhaps more desirable because any medium may be used without the addition of a buffer.

The use of gas from a laboratory jet not only reduces the cost of the set-up to about one-third its former price, but there is no need for a hydrogen cylinder or generator. Illuminating gas is less explosive and is used at less than one-twentieth to one-fiftieth of the hydrogen pressure.

In order to determine whether this method would be applicable in other cities where the municipal supply is somewhat different from that in Baltimore, analyses of supplies in various sections of the United States were obtained; samples were secured from some of them and used in the jars. If properly used, good growth should be obtained from any gas where the analysis is similar to those shown in Table I.

This anaerobic system, using illuminating gas, is being employed successfully in several laboratories in Johns Hopkins Hospital and Medical School, and in one commercial laboratory a large number of sterility tests are handled in this way very inexpensively as compared with former methods. A control tube containing *Cl. novyi* in 1:100,000 dilution is included in each jar, and good growth in forty-eight hours demonstrates the absolute anaerobiosis in the jar.

The equipment shown in Fig. 1 illustrates the usual arrangement, the cost of which is less than twenty-five dollars. The effort and time (three to five minutes) which is usually required to place the tubes in the jar, roll the plasticine seal, partially evacuate and attach the gas, may be lessened if a three-way stopcock is attached to the vacuum (B) and gas (C), and then to the jar outlet (A). After closing the jar, it is only necessary to plug in the current (110 volts through a 32 C.P. carbon filament lamp in series). When about a 50 cm. vacuum is reached, the stopcock is turned so that the gas will flow into the jar. After thirty to forty-five minutes the valve at (A) is closed, and the gas and electric currents discontinued.

The use of electrically heated catalyzer systems for the detection of anaerobes in products in ampoules and other preparations has several advantages over many other methods which are satisfactory for the less strict anaerobes, such as *Cl. sporogenes*, *Cl. welchii*, and *Cl. tetani*. One of these advantages is that in testing the sterility of biological products a large number of tubes may be tested at one time, and not only will the anaerobes which have a comparatively high oxygen tolerance be picked up, but the most fastidious organisms as well, many of which are entirely overlooked when Smith tubes or similar methods are employed. Another advantage is that absolute anaerobiosis exists from the time the jar is placed in the incubator and is not dependent on slow working catalyst or vegetable tissue systems, in which forty-eight hours may elapse before an anaerobic state is reached.

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THE WEIGHT CURVE COMPLICATED BY DEATHS*

R. D. Templeton, and Mary C. Patras, Chicago, Ill.

IF ONLY the surviving animals are considered in calculating an average weight curve, the death of individual animals frequently gives false implications to the results. For example, the average weight of four animals (Table I) was 175 gm. at a time when one animal was much smaller than the others. The death of this small animal and the dropping of it from the weight curve caused an apparent average gain in weight of 18 gm., when actually all of the surviving animals lost weight. This apparent gain is due to the fact that the dropping of one animal from the total of four is a reduction of 25 per cent in the number of animals, while the withdrawal of 100 gm. (i.e. the weight of the discarded animal) from the total of 700 gm. (i.e. the total weight of the four animals) is a reduction of only 14.2 per cent.

Table I

	Number of Animals	Weights Before Deaths	Number of Animals	Weights After One Death	Changes in Weight
	1	200	1	195	−5
	1	200	1	190	−10
	1	200	1	195	−5
	1	100	0	0	
Totals	4	700	3	580	
Averages		175		193	

To eliminate the objection referred to, an experiment may be considered as a unit consisting of several parts, each part a vital constituent until the ultimate end, the ultimate end of such an experiment being the death of all its parts. If an experiment is considered as a unit, no individual animal can be discarded before the termination of the unit. If the animal is to be considered throughout the course of the experiment, obviously its weight must be considered also.

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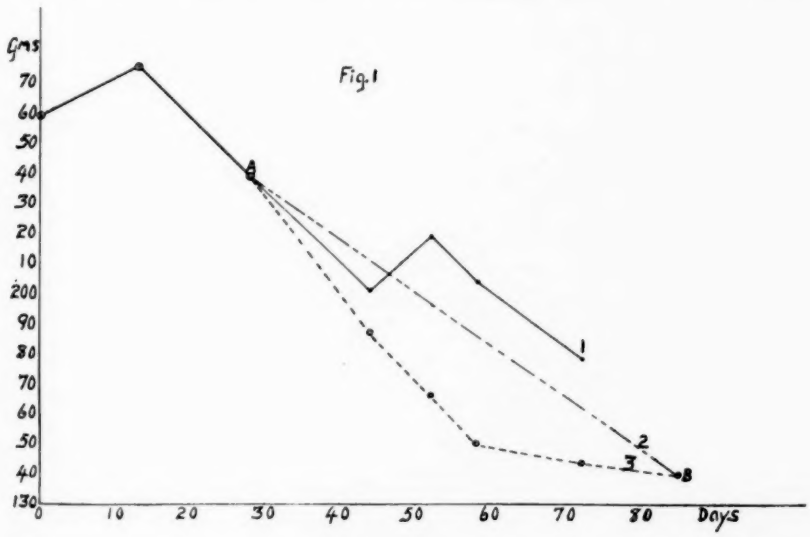
The death of an animal, although terminating it as an individual, does not eliminate it from the experiment, and, therefore, its weight at death must be carried as a part of the total weight of the unit. By using the total number of animals in the unit as the divisor and the total weight of the unit as the dividend, a curve is obtained which represents the weight of an average animal and is parallel to the curve representing the total weight of the experimental unit.

The fallacies of the weight curve where only the surviving animals are considered are especially noticeable when dealing with adult animals on a deficient diet. One of our studies in vitamin B insufficiency included 36 adult male albino rats, which prior to the experiment received a stock diet of Fox Chow ad libitum, with bread, meat, and cabbage once per week. From the start of the experiment these animals were given a diet consisting of casein, 18 per cent;



starch, 57 per cent; butter, 15 per cent; baker's yeast, 5 per cent; cod liver oil, 2 per cent; and inorganic salts (Harris), 3 per cent. They were weighed at regular intervals during the course of the experiment.

When only the surviving animals were considered in calculating the average weight of each unit (Fig. 1, curve 1), the curve seemed significant for the first forty-four days. During the first thirteen days there was a small gain in the weight, indicative of some vitamin B having been stored in the tissues of the animals while on a good diet. During the next fifteen days a decline was observed in the weight, which indicated a diminution in the stored vitamin B to the extent that an inadequacy for the well being of the animals had developed. During the succeeding sixteen days (twenty-eighth to forty-fourth) 7 animals died; however, the diminution in the weight of the surviving animals was sufficient to also show a marked decrease in the weight curve. Between the forty-fourth and fifty-second day 15 animals died. The discarding of these

animals from the experiment was relatively much greater than dropping the weight of the discarded dead animals from that of those surviving. Thus an apparent gain in weight for the average surviving animal was indicated, even though there was actually a significant loss of weight.

The last living weight obtained in the experiment was on one rat on the seventy-sixth day. The weight of that animal was 178 gm. This is the implied average terminal weight. The implication is false since this is only the weight of an individual animal, and the average final surviving weight of all the animals would be entirely different.

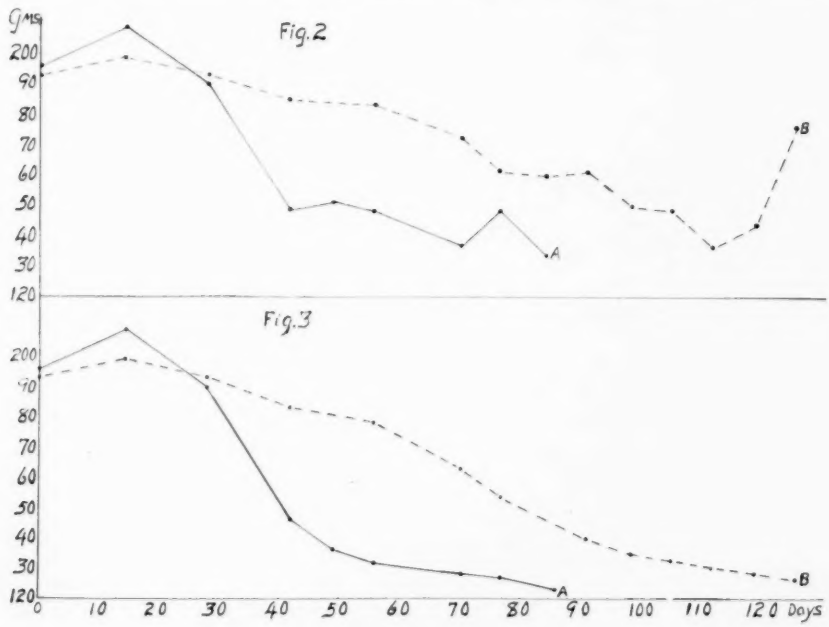
In dealing with weight data of this kind obviously there are four important points; others may appear after a more detailed study. (1) The weight of the entire unit at the start of the experiment. (2) The weight of the unit at the time of greatest gain. (3) The weight of the unit just prior to the first death. (4) The weight of the unit at the end, when all animals are dead. If the weight of the unit just before the first death (Fig. 1, point A) is significant and the dead weight of the unit (point B) is significant, then a line joining these two points must be important. A straight line joining these points (curve 2) would imply a uniform rate of weight loss for the unit, which is not true. Obviously an accurate determination of the weight between these two extremes (where all animals are living or all are dead) would include the weights of some living and some dead animals. Calculation of the average weight of the animals in the unit by this method gives a curve which reveals a rapid decline in the weight of the unit between the twenty-eighth and fifty-eighth days. This was also the period of greatest mortality in which the unit was rapidly yielding to the vitamin B insufficiency. Between the fifty-eighth and eightieth day the decline in weight was greatly decreased. The few surviving animals were near the average death weight by the fifty-eighth day, but probably were utilizing the small amount of vitamin B in the diet very economically.

A further study of the relative value of the two methods was made by applying the principles involved to a study of the growth obtained on two groups of animals, one of which (22 animals) received a vitamin B deficient diet and the other (22 animals) received a diet deficient in both vitamin B and inorganic salts. The vitamin B deficient diet was identical with the one already given in this paper. The diet deficient in both vitamin B and inorganic salts was simply a modification of the first diet in which starch was substituted for the inorganic salts.

The same general impression is obtained whether only the surviving animals (Fig. 2) are considered or the weight curve made to represent all animals whether dead or alive (Fig. 3) until the close of the experiment. The flattening of the weight curve (Fig. 2, curve A) beginning on the forty-second day of vitamin B deficiency is exaggerated when only survival animals were considered. This exaggeration is due to the death of some light animals and their withdrawal from further consideration. The sudden drop in the weight curve just

preceding the last weigh day is due to the death of one animal and its withdrawal from the group. The weight of this animal was a much greater portion of the total weight of the surviving animals than it, as an individual, was of the total number of surviving animals.

The weight curve for the group of animals receiving both the vitamin B and salt deficient (curve B) diet presents several irregularities due to death of individuals and their withdrawal from the group between the fortieth day and the close of the experiment. The large rise in the curve between the one hundred and twelfth and one hundred and twenty-sixth day is due mainly to the death of 5 animals and their withdrawal from the experiment. In this case

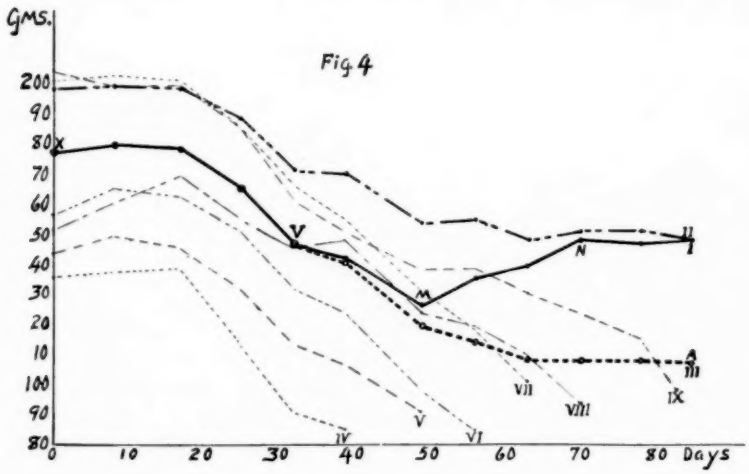


the number of animals was relatively a greater portion of the surviving animals than was the weight of these animals of the total weight of the surviving animals.

Considering the groups of animals as two units and carrying the weights of all animals, whether dead or alive (Fig. 3), to the close of the experiment gave smooth curves. These curves represent the condition of each experiment as a whole. It can then be said with some degree of certainty that from a study of 22 rats on a vitamin B deficient diet, the average death weight of similar animals on a diet similarly deficient in vitamin B should be about 123 gm. By the same reasoning it may be said that an individual having all the characteristics found in the 22 rats used here should approximate this weight curve if subjected to similar conditions. This is the usual meaning of the term "average curve."

A more detailed study of the methods applicable to determining weight curves was made on data obtained from animals during the first eighty-five

days of experimental hyperthyroidism (Fig. 4). The diet used to produce the hyperthyroidism consisted of casein, 18 per cent; starch, 46.9 per cent; unsalted butter, 15 per cent; baker's yeast, 15 per cent; cod liver oil 2 per cent; inorganic salts (Harris), 3 per cent; and desiccated thyroid, 0.1 per cent. Thirty-four rats (17 males and 17 females) which varied in age from 177 to 288 days were used. At the start of the experiment the lowest weight (135 gm.) was that of a female. The highest weight (214 gm.) was that of a male. The average weight of all animals was 177 gm. Between the beginning and the thirty-second day of the experiment there were no deaths. During the first few days some of the animals gained and others lost in weight. The curve X to V represents the average weight of all animals before deaths began to complicate the data. Curve I represents the average weight of the animals surviving on the various weigh days. The rapid rise in this curve from M to N indicates that the



surviving animals for some reason were rapidly gaining in weight. As a matter of fact, the average weight of the 9 animals which survived the entire eighty-five days did not at any time show a significant increase in weight as revealed by curve II. The rapid rise in curve I from M to N is due to the withdrawal from the weight curve of 23 animals which died between the forty-ninth and seventieth day of the experiment. This number of animals was relatively a much larger portion of the total number of animals living on the forty-ninth day than was their weight of the total weight of the living animals.

Curves IV to IX represent subgroups of animals which were recorded dead at various times during the course of the experiment. The last weight in each curve is the average death weight of the animals represented. Curve III (X-V-A) is the weight curve of the entire group whether dead or alive. Curves III and I coincide from X to V since during this period there was only a change in weight, unaffected by deaths. From V to A, however, in curve III the experiment was considered as a unit, and the dead weight of all animals which died in the course of the experiment are carried in the curve. In this

curve there are no appreciable fluctuations which are not significant. There is a gradual decline in this curve between the seventeenth and the sixty-third day, which is the general story seen in the average curves of all subgroups of animals whether they were dead on the thirty-ninth day or still living at the close of the experiment on the eighty-fifth day. This curve also shows that between the sixty-third and the eighty-fifth day there was only a slight change in the average weight of the unit which is in accord with curve II representing the subgroup which lived the entire eighty-five days.

SUMMARY

1. A weight curve which represents the average weight of surviving animals from time to time in the course of an experiment is criticized.

2. A method is proposed in which all animals on a given experiment are considered as a unit, and no individual animal is disregarded from the weight curve because of death in the course of the experiment.

COMING MEETINGS

American Society of Clinical Pathologists, San Francisco, June 9, 10, and 11. The meeting on June 9 is devoted entirely to a tumor seminar under the direction of Dr. Lee McCarthy of Washington, D. C. The subject is "Pathology of the Skin." Headquarters, Palace Hotel.

The Association for the Study of Allergy, June 10 and 11. Headquarters, Hotel Empire, San Francisco.

The American Rheumatism Association, June 13 at Toland Hall, The University of California Hospital, San Francisco.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

DIPHTHERIA, Duration of Immunity Achieved by Various Methods, Park, W. H. J. A. M. A. **109**: 1681, 1937.

There is a great deal of controversy in the literature concerning the best method of immunization against diphtheria from the point of view of duration of the immunity achieved.

None of the material available at present on this subject is suitable for the deriving of statistically valid conclusions.

The author believes that a small but very important piece of work done on animals by Dr. Povitzky suggests that the best results with two doses might be obtained by the use of a 1 c.c. dose of alum-precipitated toxoid, followed by a 1 c.c. dose of fluid toxoid.

Pending conclusive determination of the best method of immunization against diphtheria, the administration of two or three doses of either fluid or alum-precipitated toxoid is the wisest procedure.

KIDNEY, Pathology of Renal Papilla: Relationship to Renal Calculus, Randall, A., Eiman, J. E., and Leberman, P. R. J. A. M. A. **109**: 1698, 1937.

Calcium plaque formation of the renal papilla was observed in 140 of 609 autopsies, i.e., in 22.9 per cent.

In the same series of autopsies forty-nine renal papillae were observed with stone adherent thereto, and in practically every instance the stone was growing from a calcium deposition in the papillary wall.

Stone was found in twenty-five of the 609 autopsies, or in 4.1 per cent.

Our efforts to reproduce this calcium plaque formation in lower animals by vitamin-deficient diets failed.

A staphylococcus toxoid was concentrated by the rabbit's kidney from two and one-half to sixty times its blood plasma content.

The administration of a stable streptococcus hemolysin leucocidin effects highly suggestive results, causing local damage to the epithelium of the collecting tubules.

After the administration of parathyroid extract to dogs for six months, we observed in one renal papilla a calcium plaque identical to that which we have seen in man.

We wish to point out again that the occurrence of renal calculus in man is essentially only a symptom of some underlying pathologic condition of a renal papilla and that its entire development is a slow, chronic process. Acute results obtained in experiments on animals are not comparable to the clinical picture.

TUBERCULIN, A New Patch Test, Vollmer H., and Goldberger, E. W. Am. J. Dis. Child. **54**: 1021, 1937.

A new tuberculin patch test is described. The patch consists of a strip of adhesive tape on which a square of filter paper saturated with tuberculin has been placed.

Of 209 tuberculous children, 187, or 89.5 per cent, showed conformity between the results of the patch test and those of the Pirquet test. Of the 22, or 10.5 per cent, in whom the results did not conform, 15 were children who showed a positive reaction to the patch test and a negative reaction to the Pirquet test.

The patch test is not only as accurate, numerically, as the Pirquet test, but shows the following definite advantages over all tuberculin tests:

1. It is painless and does not excite nervous children, and a good relation between the doctor and the patient is thus retained.

2. It consumes less time.

3. It does not involve the use of instruments and their sterilization or trauma to the skin through injection, scarification or rubbing.

4. It can be carried out without help, or by a nurse or an assistant.

5. In contrast to all other patch tests, it creates a sharply limited area of reaction and prevents an uncontrollable spread of tuberculin on the skin.

6. It eliminates the danger of infection.

7. It has never led to a local or general constitutional reaction.

PNEUMONIA, Due to B. Friedlanderi, Bullowa, J. G. M., Chess, J., and Friedman, N. B. Arch. Int. Med. **60**: 735, 1937.

A report on 41 patients with acute pneumonia due to *B. Friedlanderi* is presented in an attempt to portray the disease as a clinical and pathologic entity. The findings for these patients showed a marked similarity to those for patients with pneumococcic lobar pneumonia.

The mortality rate for the entire group of 41 patients was 83 per cent. The highest mortality rate (94 per cent) occurred in patients infected with *B. friedlanderi* A who were not given serum.

Serum therapy was attempted for 8 of the patients, but in only 6 of them was the serum specifically related to the infecting organism. The mortality rate for these 6 patients who were infected with *B. friedlanderi* A and who received serum was 50 per cent, as contrasted with a mortality rate of 94 per cent for the 18 patients infected with the same organism and not treated with serum.

Note: Since the preparation of this report Solomon has published reports of 5 patients from Bellevue Hospital. Only 1 patient was treated as early as the third day. The 4 others were treated on the fifth day or later. The amount of serum given was inadequate. All his patients who received serum died.

HYDATID DISEASE, Godfrey, M. F. Arch. Int. Med. 60: 783, 1937.

In the course of a thorough review of this condition the author presents the following avenues of laboratory diagnosis: Three laboratory tests are of great value:

1. *The Precipitin Test.*—This test, which employs the serum of the patient in prepared hydatid fluid, is accurate in 65 per cent of cases. It is of value only when used with carefully prepared controls.

2. *The Intradermal Test.*—The intradermal test of Casoni is very valuable. It is carried out in a manner similar to that of the Pirquet test for tuberculosis. Hydatid fluid obtained by puncture of an aseptic cyst from the lung or liver of a sheep is filtered and used for this test. When 0.3 c.c. of the fluid is injected intradermally into the arm of the patient, a wheal is raised that is about 8 mm. in diameter. An injection of physiologic solution of sodium chloride should be used as a control. When the reaction is positive, the wheal reaches its maximum size of 4 or 5 cm. in from ten to twenty minutes and is surrounded by an erythematous zone. This test, according to Fairley, showed positive results in 56 per cent of the cases in which there were uncomplicated cysts and in 26 per cent of the cases in which there were ruptured or suppurating cysts. The Casoni test is a useful preoperative guide, since it indicates when there has been a dangerous degree of absorption of hydatid fluid.

3. *The Complement Fixation Test.*—This test is specific and is dependent on a specific antibody in the serum of patients who have absorbed hydatid antigen. Fairley's technique is similar to that of the Wassermann test. According to Fairley and Williams, in 52.4 per

cent of cases there is a positive reaction preoperatively; whereas in 52.8 per cent of cases there is a positive result when there are residual or recurrent cysts.

Leucocyte Count.—When the common causes of eosinophilia, such as asthma, chronic disease of the skin and intestinal worms can be eliminated, a certain amount of reliance may be placed on the presence of eosinophilia. In about half the cases of hydatid disease the eosinophile count is higher than normal, the upper limit of normal being placed by Fairley and Kellaway at 300 eosinophilic leucocytes per cubic millimeter of blood (6 per cent). Rarely, the eosinophiles outnumber the neutrophiles.

Characteristics of Hydatid Elements.—The hydatid fluid is clear, limpid, and alkaline, with a specific gravity of from 1.008 to 1.015. It contains about 6 per cent of sodium chloride. After centrifugation it should be examined for scolices and hooklets. A scolex measures 160 microns in length and 115 in breadth. Hooklets also may be seen, their length varying from 20 to 40 microns.

BACTERIEMIA, Significance of Hemolytic Streptococci, Keefer, C. S., Ingelfinger, F. J., and Spink, W. W. Arch. Int. Med. 60: 1085, 1937.

From the clinical study of 246 patients with bacteriemia the following facts were elicited:

Bacteriemia in hemolytic streptococcic infection is seen most often in patients in the first, fourth, and seventh decades. These peaks of incidence correspond to the age incidence of infections of the throat and middle ear, the puerperal infections, and cellulitis and erysipelas.

The general fatality rate was 72 per cent. It was highest for the patients with cellulitis and erysipelas regardless of age, slightly lower for those with puerperal sepsis, and lowest of all for patients less than 20 years of age with infections of the throat, middle ear, and mastoid. Aside from the differences in mortality according to age and portal of entry, such factors as the duration of the sepsis, the location of infection in an area that could or could not be treated adequately, and the presence or absence of debilitating diseases were of importance in determining the outcome.

The commonest metastatic lesions were in the joints, subcutaneous tissues, and endocardium, although only about 30 per cent of the patients showed metastases.

Recovery following bacteriemia occurred after a transitory invasion of the blood from a focal lesion without metastases or when the blood stream was cleared of organisms and focal infection was established if the lesion could be treated surgically.

Death occurred among those with debilitating diseases, those with rapidly spreading infection without localization, and those in whom localization occurred in an area which could not be treated surgically (peritoneum, endocardium, or meninges). Sometimes the blood was cleared of organisms, but the foci of infection were situated so that they could not be treated adequately.

From a study of the organisms which cause bacteriemia and the immune reactions of patients with and without bacteriemia, the following statements are justified:

Organisms isolated from local lesions and from the circulating blood were beta-hemolytic streptococci. They all belonged to group A of Lancefield, and they all produced fibrinolysin.

Organisms isolated from the circulating blood frequently resisted phagocytosis and were not killed by blood from normal persons. There are exceptions to this observation, but, by and large, it is true.

Organisms isolated from local lesions were phagocytosed by the leucocytes of some normal persons and killed in varying numbers by those of different persons. The blood of patients with local lesions usually possesses the capacity of killing the homologous organisms, and this function increases with the course of the disease. The presence of circulating antibodies assists in localizing the infection and in preventing bacteriemia.

In patients with bacteriemia antibodies develop which aid in the clearing of the blood stream. In the author's experience all the patients with bacteriemia who recovered had demonstrable antibodies in the circulating blood, and it was shown that antibodies were present in some of the patients who died, although the titer was low.

Bacteriemia in hemolytic streptococcic infection is of greater value in prognosis than in diagnosis, and its presence may be taken as an indication of a loss of equilibrium between the local defense mechanism and the normal clearing mechanism. The presence of specific antibodies plays an important part in preventing bacteriemia and in clearing the blood of organisms once it has become invaded by organisms from the local focus.

HODGKIN'S DISEASE, The Etiologic Relation of the Eosinophile to the Gordon Phenomenon, Turner, J. C., Jackson, H., Jr., Parker, F., Jr. Am. J. M. Sc. **195**: 1, 1938.

In a series of 11 cases of Hodgkin's disease the Gordon test has been positive only when eosinophiles could be found in the corresponding microscopic sections. The number of these cells was found to parallel the rapidity of development of the pathogenic substance upon which the positive test depends.

The pathogenic agent described by Friedemann and Elkeles in myeloid tissues has been shown to be present only in those suspensions of leucocytic cream that contain more than a certain number of eosinophiles.

Gordon's agent and Friedemann's agent would both, therefore, appear to be derived from the eosinophile and consequently are probably identical.

The Gordon test is positive in Hodgkin's disease only by virtue of the presence of eosinophiles in the lesions of this disorder.

PNEUMONIA, LOBAR, in Children, Andrews, E. T. Am. J. Dis. Child. **54**: 1285, 1937.

Comparison of numerical with causative frequencies of types of pneumococcus which have been recovered from children with lobar pneumonia led to their separation into three etiologic groups, the distribution of which among patients with and without pneumonia and among other members of the same families indicated a corresponding diversity in their epidemiologic significance.

A types appeared causative in all patients with lobar pneumonia from whom they were recovered, and they were associated with a relatively high incidence of pneumonia in infected persons, high mortality among infants, marked evidence of familial dissemination when the concurrent pneumonia was ascribed to these types and relatively low incidence in families without pneumonia or in those in which the patients did not show the same types of pneumococcus.

Intermediate or B types appeared causative in many, but not all, of the children with lobar pneumonia from whom they were recovered but, in comparison with the former group, showed a lower incidence of this disease in infected persons, lower mortality among infants, less evidence of familial dissemination when the concurrent pneumonia was ascribed to these types, and a relatively high incidence in families without pneumonia or in those in which the patients had pneumonia ascribed to heterologous types.

The least active types (group C) appeared to be responsible for lobar pneumonia in few of the children from whom they were recovered. Evidence for their familial dissemination was uncommon but these were the types most often recovered from persons coming in contact with patients whose pneumonia was ascribed to heterologous types.

The incidence of pneumococci, the occurrence of lobar pneumonia in infected persons, and the familial dissemination of causative types also varied with age.

Children exposed to pneumonia ascribed to A types yielded pneumococci found in the patients relatively more often than did adults.

Patients with pneumonia ascribed to A types constituted the most active sources of infection by contact.

Repeated cultures of material from the throat were helpful in revealing the probably causative types in children with lobar pneumonia.

YAWS, Comparative Histology of Syphilis and, in Jamaica, Ferris, H. W., and Turner, T. B. Arch. Path. **24**: 703, 1937.

The initial lesion and the frambesiomas of yaws are characterized by epithelial hyperplasia with exudation of polymorphonuclear leucocytes. There are many lymphocytes and

plasma cells in the dermis. The scaly macules and folliculopapules have little proliferation of epithelium and only scant infiltrations of lymphocytes and plasma cells in the dermis. In the late lesions of yaws there is usually ulceration, with varying degrees of epithelial hyperplasia.

With silver preparation, the treponemes of yaws (*T. pertenue*) are constantly found in large numbers in the epidermis and more superficial dermis of the frambesiform lesions and may be numerous in the initial lesion. They are demonstrated in small numbers in the hair follicles and dermis of about 50 per cent of the folliculopapules and scaly macules of yaws. They are occasionally recognized in small numbers in late lesions.

Spirochetes are demonstrable in about 70 per cent of the papules and maculopapules of syphilis.

There is no evidence that in either yaws or syphilis the spirochetes are phagocytosed by polymorphonuclear leucocytes or histiocytes.

There is no correlation between the titer of the Wassermann reaction of the blood and the presence or number of spirochetes in the cutaneous lesions of yaws.

Histologic criteria for the differentiation of the cutaneous and subcutaneous lesions of yaws and syphilis are in general unreliable.

SHELL-FISH POISONING, Paralytic. Sommer, H., and Meyer, K. F. Arch. Path. **24**: 560, 1937.

The authors have tabulated and discussed 243 cases of paralytic shellfish poisoning, with sixteen deaths, that occurred between Ventura County, Calif., and Juneau, Alaska, from 1927 to 1936. Of these, 234 cases were caused by the coast mussel; 9 were caused by the Washington clam.

Methods for detecting and quantitatively estimating the poison in shellfish have been worked out. Mussels from numerous places between central California and southern Oregon have been analyzed and toxin curves constructed for the past nine years. The curves for mussels, sand crabs, and Washington clams from localities near San Francisco since 1931 have been specially detailed. Besides the shellfish mentioned, seven of the common varieties of edible clams may contain the poison in smaller amounts.

Poisonous mussels may in no way be distinguished from normal ones, except by the animal test. Mussels subjected to various conditions in the laboratory have never shown an increase in toxicity; they usually show detoxification, the rate of which has been determined. Mussels may take up poisons from sea water.

Strong evidence has been presented which points to the water of the open ocean as the carrier of the poison. Owing to the strong adsorption of the substance on base-exchanging silicates of the sand, it is not likely to occur free in the water. The poison has been demonstrated, at least during the poison season, in the residue from filtration of sea water. Whether it is contained in the plankton or adsorbed in the microscopic sand cannot at present be decided.

Four different principles toxic for mice have been demonstrated in acid alcoholic extracts of shellfish and plankton. The methods follow:

Standard Test.—The shellfish are opened by cutting the posterior abductor muscle, the digestive glands are carefully removed and without the addition of shell water or of other tissues, are ground up thoroughly with acid methyl alcohol (4 c.c. of concentrated hydrochloric acid per liter). Several portions of the liquid are used, so that the residue becomes nearly colorless. The combined residue and liquids, which measure approximately from 60 to 80 c.c., are poured into a centrifuge tube. The mixture is brought to a boil by immersion of the tube in hot water and is kept boiling for a few minutes. Next it is gradually cooled to room temperature, preferably in the same water-bath. (If necessary the extract may be cooled rapidly and worked up at once, or it may be stored for several days. It should, however, not be kept longer than a week.) After standing for a few hours or overnight, most of the alcohol-insoluble substances have settled; they are removed by centrifugation, and the clear dark-colored fluid, or an aliquot part, is poured into a weighed glass dish and

evaporated on a boiling water-bath. The amount of fluid chosen depends on the time taken for evaporation, i.e., not more than from thirty to forty-five minutes, and the amount of residue expected; 200 mg. is sufficient for a test; from 50 to 200 mg. is usually obtained from a mussel. The residue is removed from the water-bath before it is entirely dry, i.e., when it is still of a pasty consistency. It is triturated with the help of a glass rod, with several small portions of chloroform, until the latter comes off nearly colorless. The solvent is poured off each time and discarded. This extraction of the lipids and pigments may be readily accomplished if the residue shows the degree of dryness mentioned. Floating particles may, however, be made to settle by the addition of ether. The residue is next heated on the water-bath for one or two minutes, with constant stirring, until it is quite viscous. It is hygroscopic and should be weighed as soon as cold. For the injections distilled water ten times the weight of the residue is added, making a solution of approximately 10 per cent.

It is evident that the method can be modified and adapted to specific needs. For the detection of mere traces of poison it may be considered advantageous to evaporate in vacuo; better yet, the poison may be precipitated from the alcoholic solution by the addition of from 5 to 10 volumes of ether. The dried residue may then be directly dissolved for injection. On the other hand, certain errors must be avoided. Grinding of the tissue with sand, as performed in 1927, is of decided disadvantage, since it has been shown that the shellfish poison is adsorbed by sand in much the same way as by permutit (an artificial sodium aluminum silicate). If large amounts of extract have to be injected, so that it becomes desirable to neutralize the solution, great care must be exercised in doing this, since even a slightly alkaline reaction may destroy 50 per cent of the poison in a short time. In the standard test as done at present the solution is not neutralized for injection. The thorough extraction with chloroform and the subsequent heating should be ample to remove the free hydrochloric acid introduced. It is very likely that the remaining acid is bound to betaine which is found in large amounts in any extract of shellfish and which is quite harmless on intraperitoneal injection in the form of its hydrochloride. A fatality from the acid reaction alone has never been encountered.

The average lethal dose of shellfish poison has been defined as that amount which will kill a 20 gm. mouse in from ten to twenty minutes, with typical neurotoxic symptoms. Besides uneasiness, a wobbling gait, strong spasms and gaspings, the heart-block may be used as a differential symptom. It is present only when the dose of poison is not too massive; otherwise, the heart is entirely stopped. It develops soon after the heart has been exposed and may last for as long as half an hour. The intestinal peristalsis also is invariably pronounced on autopsy immediately after death from pure mussel poison.

Field Test.—Another test for shellfish poison, which is of great help in routine examinations, consists in extraction of the livers with boiling acidulated water, cooling, and injection of 1 c.c. of the supernatant liquid. It has been used as a field method when rapid examination of several mussel beds became imperative for the establishment of quarantine measures. For an average-sized mussel liver, 1 c.c. of tenth normal hydrochloric acid plus 9 c.c. of water was used. Thorough grinding of the tissue is essential in this test also. One minute of boiling is usually sufficient to coagulate the bulk of the insoluble material; the acid need not be neutralized before injection. Owing probably to the inclusion of poison in the precipitated protein, this test shows about half the number of average lethal doses per mussel expected according to the standard test. The ratio is still more unfavorable when neutral extracts are compared.

REVIEWS

Books and Monographs for Review should be sent direct to the Editor,
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

A Textbook of Hematology*

THIS volume presents a concise but fairly comprehensive discussion of normal and abnormal hematology and a discussion of the more important diseases of the blood.

While the author states in his preface that the purpose of the book is "to present the subject of hematology in a manner acceptable to practising physicians as well as to those primarily interested in the study of disease by laboratory methods," his book will appeal to the physician rather than to the clinical pathologist or hematologist already familiar with the subject.

The chapter on laboratory technique is too brief to add materially to the value of this text, and while their application to the study of anemias is obvious, tests for free hydrochloric acid in gastric contents, urobilin in the urine and the occult blood in the feces appear somewhat out of place in a book concerned with hematology. The general character of the book is such as to indicate that it was prepared primarily with the clinician in view, and to those who are desirous of becoming informed upon the present views of disorders of the hematopoietic system it should prove of interest and value. It cannot, however, be regarded as an exhaustive discussion of the subject.

Pulmonary Tuberculosis in Practice†

THIS is a very stimulating book. Though small, it contains a wealth of information reflecting an obviously extensive experience thoughtfully digested.

Dr. Wingfield's book is written for the practitioner at large and should receive a cordial welcome. Starting with the pertinent observation that, though the common tendency is to refer the tuberculous patient to specialized hands, the diagnosis and perhaps the most important part of the treatment fall within the province of the general practitioner, Dr. Wingfield attempts to bridge the gap between the practitioner and the specialist.

In the first part of this book he traces the life history of the tuberculous lesion in the lung from its initial appearance through its possible stages of development to its finish. In the second part of the book the corresponding clinical pictures are described and the appropriate methods of treatment discussed in detail where they concern the practitioner, broadly where they fall within the province of the specialist.

A large and well planned diagram divides chronic pulmonary tuberculosis into twelve stages in one of which the patient will fall, whether presenting himself for diagnosis or returning to the practitioner after institutional treatment.

It is impossible to read this book without profit and it can be recommended as a well-balanced thoughtful study based upon an ample and well-digested experience.

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*A Textbook of Hematology. By William Magner, M.D., D.P.H., Pathologist, St. Michael's Hospital, Toronto, Canada, Lecturer in Pathology, University of Toronto, Formerly Lecturer in Pathology, University College, Cork, Ireland. Cloth, 395 pages, 23 figures, 3 colored plates, $4.50. P. Blakiston's Son & Co., Inc. Philadelphia, Pa.

†Pulmonary Tuberculosis in Practice. A Modern Conception. By R. C. Wingfield, Medical Superintendent, Brompton Hospital Sanitorium, Frimley; Formerly Tuberculosis Office, St. Thomas' Hospital. Cloth, 122 pages, 25 figures, $2.50. William Wood & Co., Baltimore, Md.